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# BRONCHIAL HYPERRESPONSIVENESS AND ITS RISK FACTORS IN FINNISH ADULT POPULATION

**by**

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ACADEMIC DISSERTATION

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Air roots by Taru Tolppo

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*Minä kurotan maahan,*
*ja juurrun taivaalle,*
*yhdessä syvässä henkäyksessä.*

– Ilmajuuret, T.Tolppo –

*I reach for the ground,*
*and I root myself to the sky,*
*in one deep breath.*

– Air roots by T.Tolppo –

# ABSTRACT


**Aim**

The main aim of the study was to assess the prevalence of bronchial hyperresponsiveness (BHR) in the Finnish adult general population, its determinants and risk factors. First, the two most commonly used BHR methods, histamine and methacholine challenges, were compared to assess their agreement. Secondly, prevalence of BHR, its risk factors, and the association of active and passive smoking with BHR severity was studied in detail. Incident adult asthma in Helsinki over the 11 years' follow up was investigated.

**Subjects**

For the BHR methodological comparison study 79 subjects (21-73 years) were included in Kemi, Finland, whereas 292 randomly selected subjects (26-66 years) were included for the Helsinki BHR-study. The follow up study of incident asthma and respiratory symptoms in 2007 included 4302 replies (participation rate 79%) of those, who had originally (n=6062) taken part in the FinEsS-Helsinki postal survey in 1996.

**Methods**

In Kemi, following the postal survey, subjects were invited for clinical lung function tests. Bronchial challenges to methacholine and histamine were performed to each subject in a randomized order. In Helsinki, following the interview, skin prick tests, spirometry, and fractional exhaled nitric oxide (FENO) measurement, the BHR test with histamine was assessed. Provocative doses for histamine and methacholine were assessed ($PD_{15}FEV_1$ and $PD_{20}FEV_1$) and dose response ratios (DRR) were calculated. The highest cumulative dose for methacholine was 2.6 mg, and the highest non-cumulative dose for histamine was 1.6 mg.

Histamine $PD_{15}FEV_1$ 0.4 mg (marked BHR, $BHR_{ms}$) and 1.6 mg (BHR) served a cut-off points in the logistic regression analysis. Prevalence and incidence of asthma and respiratory symptoms were defined, and risk factors for BHR and incident asthma were determined.

**Results**

The agreement of the histamine and metacholine challenge methods was 80% (kappa 0.45; 95% CI 0.23-0.68) in the study cohort of individuals without physician diagnosed asthma or chronic bronchitis. In staging the severity of BHR methods, the agreement was good (weighted kappa 0.64; CI 95% 0.46-0.82).

In Helsinki, BHR was found in 21.2% of the general adult population. Severe or moderate BHR ($BHR_{ms}$) was found in 6.2% of the subjects. $FEV_1$ < 80% of predicted and airway obstruction ($FEV_1$/ FVC < 88% of predicted) yielded over four-fold risk for BHR, in which the risk increased by the BHR severity. The results indicated that smoking (OR 4.60) plays an independent and dose-dependent role in markedly increased BHR even after correction of decreased lung function. Ever smokers comprised 69% of those with BHR, and the young age of starting to smoke constituted on BHR (OR 4.03). Exposure to environmental tobacco smoke (ETS) both at work and at home increased the risk for BHR (OR 6.09). Wheezing or asthma in childhood (OR 3.66) and female gender (OR 2.14) were also independent determinants for BHR in the multivariate model. Body mass index (BMI) did not associate with BHR. The association between FENO and BHR was dependent on smoking habits. Only among the non-smokers FENO leveled the BHR severity.

In the 11 years's follow up, 157 onsets of asthma occured, corresponding an incidence rate 3.7/1000/ year. Remission was 17% during that period. We observed a high incidence rate of allergic rhinoconjunctivitis (ARC) (16.8/1000/year), especially among women, and in the youngest age groups. Allergic rhinocounjuctivitis (ARC) doubled the risk for asthma, but living on countryside or in a farm below five years of age decreased the risk (OR 0.75).

**In conclusion**

BHR tests with metacholine and histamine tests showed a good agreement in classifying severity of BHR in a population with no previous diagnosis of asthma or chronic bronchitis. Prevalance of BHR was 21%, and for $BHR_{ms}$ in 6% in the general poplation. The main determinants for BHR were a decreased $FEV_1$ and airway obstruction. Smoking and BHR were dose-dependently associated. Respiratory symptoms, asthma in the early childhood (<5) and female gender were associated with BHR, as did $MEF_{50}$ < 63% predicted.

In a longitudinal setting, our results suggested that asthma incidence in Finland has levelled on a plateau. Age >70 years, family history of asthma, allergic rhinoconjuctivitis, woman gender, and ever smoking increased the risk for adult onset asthma.

Findings of this thesis suggest that quantitative assessment of BHR by different cut off levels provides a tool for characterization of phenotypes of airway disorders in epidemiological studies.

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original communications, referred to in the text by their Roman numerals (I-IV). In addition, some unpublished data are presented.

I Juusela M, Poussa T, Kotaniemi J, Lundbäck B, Sovijärvi A. Bronchial hyperresponsiveness in a population of North Finland with no previous diagnosis of asthma or chronic bronchitis assessed with histamine and methacholine tests. Int J Circumpolar Health 2008; 67(4): 308–317.

II Pallasaho P, Juusela M, Lindqvist A, Sovijärvi A, Lundbäck B, Rönmark E. Allergic rhinoconjunctivitis doubles the risk for incident asthma – Results from a population study in Helsinki, Finland. Respir Med 2011; 105; 1449–1456.

III Juusela M, Pallasaho P, Sarna S, Piirilä P, Lundbäck B, Sovijärvi A. Bronchial hyperresponsiveness in an adult population in Helsinki: decreased FEV1, the main determinant. Clin Respir J 2012; DOI:10.1111/j.1752-699X.2011.00279.x.

IV Juusela M, Pallasaho P, Rönmark E, Sarna S, Sovijärvi A, Lundbäck B. Dose-dependent association of smoking and bronchial hyperresponsiveness in the adult general population in Helsinki. Submitted to Eur Respir J 2012.

# ABBREVIATIONS

| | |
|---|---|
| AAM | Alternatively activated macrophage |
| Ach | Acetylcholine |
| AHR | Airway hyperresponsiveness |
| AMP | Adenosine monophosphate |
| APS | Aerosol provocation system |
| ARC | Allergic rhinoconjunctivitis |
| ARIA | Allergic Rhinitis and Its Impact on Asthma |
| ASM | Airway smooth muscle |
| ATP | Adenosine triphosphate |
| ATS | American Thoracic Society |
| BHR | Bronchial hyperresponsiveness |
| $BHR_{ms}$ | Moderate or severe, i.e. marked BHR |
| BMI | Body mass index |
| cAMP | Cyclic adenosine monophosphate |
| Ch | Choline |
| CI | Confidence interval |
| COPD | Chronic obstructive pulmonary disease |
| CPET | Cardiopulmonary exercise testing |
| CRS | Chronic rhinosinusitis |
| Dg. | Diagnosed |
| DRR | Dose response ratio |
| DRS | Dose response slope |
| EAACI | European Academy of Allergy and Clinical Immonology |
| EAR | Early allergic reaction |
| EBC | Exhaled breath condensate |
| ECP | Eosinophilic cationic protein |
| EIB | Exercise-induced bronchoconstriction |
| ECRHS | European Community Respiratory Health Survey |
| ECSC | European Community for Steel and Coal |
| EPSP | Excitatory postsynaptic potential |
| ERS | European Respiratory Society |
| ETS | Exposure to environmental tobacco smoke |
| FENO | Fractional exhaled nitric oxide |
| $FEV_1$ | Forced expiratory volume in one second |
| FinEsS | Finland, Estonia, Sweden |
| FRC | Fractional residual capacity |

| | |
|---|---|
| FVC | Forced vital capacity |
| GA²LEN | Global Allergy and Asthma Network of Exellence |
| GINA | Global Initiative for Asthma |
| GM-CSF | Granulocytemacrophage colony stimulating factor |
| GTP | Guanosine triphosphate |
| GDP | Guanosine diphosphate |
| H1-H5 | Histamine receptors 1–5 |
| HDAC2 | Histone deacetylatation |
| HDM | House dust mite |
| HIST | Histamine |
| ICS | Inhaled corticosteroids |
| IgE | Immunoglobulin E |
| IL | Interleukin |
| $IP_3$ | Inositol triphosphate |
| IRR | Incident risk ratio |
| LAR | Late allergic reaction |
| LLN | Lower limit of normal |
| M1-M5 | Muscarinic cholinergic receptors 1-5 |
| MAP-study | Environ**M**ent and **A**sthma in **P**-county -study |
| MBP | Major basic prote |
| MEF50 | Maximal expiratory flow at 50% of FVC |
| Mch | Methacholine |
| NANC | Non-adrenergic non-cholinergic |
| Neb | Nebulizer |
| NO | Nitric oxide |
| OLIN | Obstructive Lung disease in northern Sweden |
| OR | Odds ratio |
| PAF | Platelet activating factor |
| PC | Provocative concentration |
| $PC_{20}FEV_1$ | Provocative concentration causing a 20% decrease in $FEV_1$ |
| PD | Provocative dose |
| $PD_{15}FEV_1$ | Provocative dose causing a 15% decrease in $FEV_1$ |
| PET | Positron emission tomography |
| ROS | Reactive oxygen species |
| RSV | Respiratory syncytial virus |
| RV | Rhino virus |
| SAPALDIA | The Swiss Cohort Study on Air Pollution and Lung Diseases in Adults |
| SD | Standard deviation |
| SPECT | Single-photon emission computed tomography |
| SPT | Skin prick test |

| | |
|---|---|
| SOB | Shortness of breath |
| TGF | Transforming growth factor |
| Th | T helper |
| TNF | Tumor necrosis factor |
| TSLP | Thymic stromal lymphopoietin |
| TER | Transepithelial electrical resistance |
| TJ | Tight junction |
| WHO | World Health Organization |

# 1 INTRODUCTION

Bronchial hyperresponsiveness (BHR) is a valid measure of the functional airway disturbance typically seen in asthma (Yick *et al.* 2012, Brannan 2010, Hargreave *et al.* 1981). BHR testing has been commonly used in epidemiological asthma studies (Cockcroft *et al.* 1977 & 1983a, Woolcock *et al.* 1987, Nowak *et al.* 1996, Burney *et al.* 1987, Norrman *et al.*1998, Toelle *et al.* 2004, Lundbäck *et al.* 2001), and in research on respiratory pathophysiology (Tiffaneau 1955, Laitinen 1974, Sterk *et al.* 1985, Ward *et al.* 2002, Downie *et al.* 2007, Chanez *et al.* 2010, Bossé *et al.* 2011).

Over the past four decades, bronchial provocation testing has been commonly used to diagnose or monitor asthma in Finland (Alanko 1970); usually followed by spirometry and bronchodilation tests. It has been feasible to include BHR testing in respiratory research protocols, in order to gain more information of ventilatory disturbances (Haahtela *et al.* 1991). BHR has been evaluated in the assessment of pathological findings from bronchial biopsies of asthmatic subjects and normal controls (Laitinen *et al.* 1985, Karjalainen *et al.* 2000, Lindqvist *et al.* 2003).

Some of the earlier Finnish BHR-studies have focused on the development of methods to assess BHR (Nieminen *et al.* 1987, Nieminen *et al.* 1988, Nieminen 1992, Sovijärvi *et al.* 1993) in order to improve the repeatability and accuracy of the BHR measures. Different ways of reporting the results of a bronchial challenge tests have been examined (Seppälä *et al.* 1990, Hedman *et al.* 1998) in order to feasibly relate the reseach data to the clinical work. However, the BHR data obtained for the general adult population in Finland remains lacking, and detailed epidemiological research to assess is warranted.

According to questionnaire studies, the prevalence of asthma was 7 % in Finland in 1996, but it has increased over the last decades (Pallasaho *et al.* 1999, Kotaniemi *et al.* 2001, Kilpeläinen *et al.* 2001). The prevalence of asthma is considered to be level with that reported in the other Nordic countries and Central Europe (Lundbäck *et al.* 2001, Bakke & Gulsvik 2000; Burney *et al.* 1994, Chinn *et al.* 1997). Recent results from the FinEsS-Helsinki cohort, however, report a 10% prevalance of physician diagnosed asthma in 2006 (Kainu *et al.,* personal communication), indicating an increase, which is in line with the reported observations from Italy (De Marco *et al.* 2012).

When assessing the long term changes in the prevalence of asthma, it is necessary also to assess the changes in the magnitude of BHR in the general population. The present BHR-studies have been carried out before the exposure to environmental smoke (ETS) in public places and restaurants became prohibited in Finland in 2006, thus providing valuable baseline data for longitudinal studies. This study

design enables the evaluation of effectiveness of the restriction of smoking exposure by following changes in the prevalence and incidence of BHR or respiratory symptoms in the general population. This work provides a much needed revision of this approach to lung function testing and its research, which has previously not received sufficient attention.

In the presented studies, bronchial challenge tests were conducted by using either a dosimetric histamine or methacholine challenge method (Sovijärvi *et. al.* 1993, Nieminen 1992). The first clinical study presented in this thesis (Study I) was assessed in Kemi in 1996-1997, and the third and fourth in a randomly selected general adult population in Helsinki, Finland in 2001-2003. The findings of the BHR tests and the rate for incidence of asthma were evaluated from replies of postal surveys (Study I and II) and a clinical interview (Studies III and IV).

The purpose of this study was to assess, for the first time, the magnitude of BHR in Finland. Employing an epidemiological study setting, the focus was to study the risk factors and determinants for BHR in the general adult population, thus creating points of interest for more specific study projects in the area of incident respiratory illness or prevention of respiratory diseases. These studies are part of an epidemiological (FinEsS) study in which, in a longitudinal setting, follow-up studies are in progress in Finland, Estonia, and Sweden since 1996.

# 2 REVIEW OF THE LITERATURE

## 2.1. OVERVIEW

The earliest documentations of research on BHR originates from the studies by Henry Salter in the 1800s, followed by studies by Alexander & Paddock (1921) and Tiffaneau (1955) (James & Ryan 1997, Yernault 1997). BHR remains, however, as an unexplained process in respiratory pathophysiology (Boulet 2003, Sinard *et al.* 2005).

In the 1980s, and at the beginning of the 1990s, several epidemiological studies reported an increased trend in the prevalence of asthma and allergy in Western countries (Jansen 1999). BHR testing was included in many of them, although mainly as a variable of lung function measurements, not as an independent outcome to consider. Family history, allergy, and smoking had already been defined as risk factors for asthma (Sunyer *et al.* 1997, Lundbäck 1998), and the "hygiene hypothesis" was gradually established based on the results of ongoing epidemilogical research (Strachan 1989, Braun-Fahrländer *et al.* 1999, Remes *et al.* 2002). The role of BHR as a sign for bronchial obstruction became more investigated in the general population (Britton *et al.* 1994). The allergy-asthma pathway from early childhood was strongly believed to precede the incidence of asthma in early years of life, or later in the teenage and adult years (Kusel *et al.* 2007).

During the past decade, action towards restoring the balance of innate and acquired immunity has been planned and executed (von Hertzen *et al.* 2009). Based on the valuable experience of the National Asthma programmes in Finland 1994–2004, and The French plan 2002–2005, the importance of reducing the number of patients with severe or moderate asthma has been acknowledged in many European countries (European Lung White Book 2003, p.22). Due to a wide body of evidence simultaneously gathered from many research laboratories, countries, and study cohorts, it has become obvious that neither asthma nor chronic obstructive pulmonary disease (COPD) are single diseases. It is hoped that this new insight will lead to better preventive and therapeutic strategies against the global burden of asthma (European Lung White Book 2003, p.21–23).

Research conducted on BHR and asthma, over recent years, has focused on the different type of inflammatory reactions and mediators (Barnes 2008, Sahlander *et al.* 2010, Marsland *et al.* 2011, Koarai *et al.* 2012). The cross linking of Th1 and Th2 defence actions (Koya *et al.* 2009), functions of the altered macrophages (Byers & Holtzman 2011), and migration of cells in the airway smooth muscle (ASM) (Tran

*et al.* 2006) have appeared to be the hottest topics. The biological function of the epithelial barrier, ASM, and the interaction of these two have been of great interest (Siddiqui *et al.* 2007, Xiao *et al.* 2011). More trials of asthma and COPD have focused on the functional disturbances of the airways, quantifying them with more advantagious techniques (Tgavalekos *et al.* 2007). The epidemiological research on asthma, allergy, and BHR has evolved to now focus on more specific genetic and epigenetic associations (Karjalainen *et al.* 2002b, Van Eerdewegh *et al.* 2002, Thomsen 2007, Renkonen *et al.* 2010, Prescott & Saffery 2011).

## 2.2. BRONCHIAL HYPERRESPONSIVENESS (BHR)

### 2.2.1. DEFINITIONS

Bronchial hyperresponsiveness (BHR) is defined as a reactive narrowing of the airways, which leads to airflow limitation (Cockcroft & Davis 2006a). BHR is typically seen in bronchial airway diseases, such as asthma and chronic obstructive bronchitis (Joos *et al.* 2003). It is one of the main diagnostic criteria for asthma (Global Initiative for Astma, GINA Guidelines, http://www.ginaasthma.org). BHR test results, however, depend on the cut off levels used, thus different sensitivity and specificity values are evident for asthma depending on the different constriction agents and methods used (Sovijärvi *et al.* 1993, Godfrey *et al.* 1999, Joos *et al.* 2003, Koskela *et al.* 2003a&b). BHR is generally considered to be present when the histamine or metchacoline $PC_{20}$ is <8-16 mg/mL or the $PD_{20}$ is <3.9-7.8 µmol (Joos *et al.* 2003), and according to the methods used in Finland (Sovijärvi *et al.* 1993, Nieminen 1992), histamine $PD_{15}$ ≤1.6mg and methacholine $PD_{20}$ ≤2.6mg, respectively.

The GINA Guidelines suggest the use of BHR testing following a normal spirometry (http://www.ginaasthma.org) for further examinations of undiagnosed respiratory symptoms. The European Respiratory Society (ERS) Task Force (Joos *et al.* 2003) recommends the use of BHR testing also in titration of anti-inflammatory theraphy. The use of BHR testing in monitoring asthma treatment has proven to lead to better asthma control, fewer exacerbations, and reduced airway inflammation (Sont *et al.* 1999, van Rensen *et al.* 1999, Lundbäck *et al.* 2008), thus it is included as an objective lung function measure to follow up asthma in many countries. The Finnish Guidelines for asthma diagnosis and treatment (Astman Käypä Hoito – suositus) consider BHR to histamine $PD_{15}$ ≤0.4 mg and methacholine $PD_{20}$ ≤0.600 mg specific for a physician diagnosed asthma, and define a 30% change in the PD-value to be a significant sign of a treatment response (Sovijärvi *et al.* 1993, Sovijärvi *et al.* 2003). In Sweden, however, no such clinical cut off levels for asthma diagnosis and treatment exists.

The degree of increased airway reaction following a natural or induced stimulus may induce a different response magnitude in healthy subjects and also in those with an airway disease, due to intra and intersubject variation in the degree of BHR (Godfrey *et al.* 1999), which demonstrates the difficulty of using BHR testing in some patients or study cohorts (Hewitt 2008). The reduced cut-off levels, for example histamine or metchacholine < 1 mg/mL, present a high specificity and positive predicted value for asthma, whereas higher cut-off levels are applicable to exclude active asthmatic inflammation due to their high value for sensitivity (Joos *et al.* 2003, Cockcroft 2010)

BHR has been traditionally divided into two components, the transient and the persistent, which provides the explanation as to why some asthmatics do not show BHR regardless of the findings of other lung function tests, and some asthmatics have BHR regardless of being treated well and being asymptomatic (Cockcroft & Davis 2006a, Busse 2010). The transient component of BHR is associated with rapid changes and reactions of the bronchus, such as exposure to allergen or occupational sensitizers. It is related to current asthma activity, and is typically the only component present in both the early stages and duration of the disease. The persistent component is believed to show features of airway remodelling and is related to both functional and structural changes in the airway due to chronic duration of the disease (Cockcroft & Davis 2006a, Cockcroft 2010).

### 2.2.2. OUTLINES OF BHR MEASUREMENTS

The quantification of BHR is impossible without measurements of airflow limitation (Yernault 1997). Bronchial provocation testing is a standardized method in the evaluation of lung function disturbances in subjects of all ages (American Thoracic Society, ATS 2000). Lung function measurements at baseline, and after an induced bronchial challenge test, form the basic structure in assessment of BHR. After bronchial provocation, a broncodilatation test is performed and the reversibility assessed (Hughes & Pride 2001, p.220-230).

BHR can be measured by different methods with varying criteria of abnormality (Rijcken *et al.* 1989, Joos *et al.* 2003, Koskela *et al.* 2003b). A serious attempt has been made to constitute the variety of the tools for measuring the BHR in the ERS (Joos *et al.* 2003) and ATS (2000) guidelines for BHR testing. Furthermore, an ERS Task Force for bronchial challenge testing is one of the ongoing projects on the international co-operation of respiratory physiologist and pulmonlogists.

In order to conduct a bronchial provocation test, a standardized method should be used (Sterk *et al.* 1993). This includes a measure to follow the induced air flow limitation, an established setting for the agent delivery (if not a free run test), a mathematical programme to calculate the final airway response, and a validated

**Table 1.** Pharmocological and physiological agents used for inhaled provocation tests, agents for a direct and an undirect bronchial challenge test.

| Direct | Indirect | |
|---|---|---|
| **Pharmacological** | **Pharmacological** | **Physical** |
| Histamine | Metabisulfite/ $SO_2$ | Exercise |
| Methacholine, ACh, cholinergic analogues | Potassium chloride | Hyperventilation |
| Prostaglandins | Propranolol | Cold air<br>Airway drying |
| Leukotrienes | Neuropeptides | Osmotic triggers:<br>hypertonic saline<br>hypotonic saline<br>distilled water |
| Bradykinin | AMP | mannitol powder |

[modified from Hughes & Pride 2001, p. 222]

way of reporting the severity of the BHR (James & Ryan 1997, Cockcroft 2010). The subject must be examined prior to bronchial provocation. There is a list of contraindications for a bronchial challenge test, including a cut off level for insufficient ventilatory function or a moderate level of airway obstruction (ATS 2000). A technician performs the test, although a physician needs to be present and available if a severe airway obstruction requires treatment. A step-wise increase of the provocative agent helps monitoring the state of airway reaction. After the bronchial provocation test, a bronchodilatation drug is given in order to resume baseline lung function capacity.

A direct provocative agent, such as histamine or methacholine, acts on airway smooth muscle (ASM) cells, which leads to a ASM constriction and narrowing of the airway calibre typical for airway obstruction. Traditionally, this ASM contraction is believed to be due to the activation of muscarinic receptors after the release of acethylcholine from the cholinergic nerve plates at the neuromuscular synapsis of the parasympathetic axons of the vagal nerves. This approach is in contrast to the indirect methods, which trigger the induced ASM contraction and airway flow limitation by causing an excess release of inflammatory mediators, such as histamine, leukotrienes, and prostaglandins, which cause a cascade that determine in ASM constriction. (Pauwels 1988, Hughes & Pride 2001, p.221-222, Van Schoor *et al.* 2000, Anderson 2010, Busse 2010) (Table 1)

Metacholine and histamine have had an inevitable position in measuring the BHR (Laitinen 1974, Cockroft *et al.*1977, Hendrick *et al.* 1986). They are the most commonly used constrictors for BHR tests in adults (Hughes & Pride 2001, p.222).

Spirometry is most commonly used measure in the assessment of induced flow limitation, because it is highly reproducible (Cochrane *et al.* 1977), the results are

applicable to the associated clinical work. Cardiopulmonary exercise testing (CPET), which includes a breath by breath analysis of ventilation and intra-breath analysis during the exercise, is an optimal method for studying symptoms typical for exercise induced bronchoconstriction (EIB) among athletes as well in patients with dyspnoea and co-morbities (Wasserman *et al.* 2005).

Delivery of the agent into the bronchus plays an important role, and is clearly defined for each of the methods in use. The three most commonly used methods for administration of inhaled pharmacological constrictor are: continuous tidal breathing of nebulized agent (Cockcroft *et al.* 1977), breath-actuated dosimeter (Sterk *et al.* 1993), and investigator-activated dosimeters, such as the Yan method (Yan *et al.*1983).

The use of inhalation syncronized dosimetric tidal breathing methods has become more popular recently, because of the high reproducibility and repeatability of this breath actuated method (Sovijärvi *et al.* 1993, Jögi *et al.* 1999, Chinn & Schouten 2005), in which the precise output dose of the agent can be calculated and the dose to the lungs evaluated (Nieminen *et al.* 1987). This method standardizes the constrictor agent's delivery, which is known to be dependent on inhalation technique (Allen *et al.* 2005, Sinard *et al.* 2005, Cockcroft *et al.* 2005, Cockcroft & Davis 2006b, Cockcroft 2008).

The avoidance of deep inhalation during agent delivery has been suggested, as it affects the final results of the bronchial challenge test (Cockcroft & Davis 2006b, Prieto *et al.* 2006). Deep inhalation may cause ASM contraction in asthmatic subjects, while in mild asthmatics and healthy subjects the effect may be opposite (Skoot *et al.* 1995, Scichilone 2001, Brusasco *et al.* 1999, Kapsali *et al.* 2000, Cockcroft *et al.* 2005). Deep inhalations also fail to cause bronchoprotective bronchodilatation in subjects with a significant BHR (Allen *et al.* 2005). Way of breathing before or during the agent delivery alter the ASM state and bronchial tone in a different way in asthmatics compared to normal subjects (Sinard *et al.* 2005). Deep inhalations taken immediately after the constricting agent have been reported to reduce the actin-myosin cross-bridges, thus altering the ASM tone (Crimi *et al.* 2008, Fredberg *et al.* 1997).

Differencies in the inhalation techiques might cause false negative findings with the dosimetric deep inhalations' method in comparison to the two-minute tidal-breathing method (Sundblad *et al.* 2000, Cockcroft *et al.* 2005, Burke *et al.* 2009). Allen *et al.* (2005) observed that a negative result was evident with the dosimetric method for half of the subjects with a $PC_{20}$ from 2mg/mL to 16 mg/mL. In contrast, Cockrcoft (2008) observed that the tidal breathing method produced twice the response (a half of $PC_{20}$) on average, of that which is reached with the dosimeter method. Similarly, Todd *et al.* (2004) showed that a dosimeter method with sub-TLC inhalations (i.e. approximately half inspiration capacity breaths) produces

substantially lower $PC_{20}$ values than the ERJ/ ATS recommended five deep breaths' dosimetric method (Sterk *et al.* 1993, ATS 2000).

Interpreting the results of BHR is method dependent. The most common way of presenting the results of the bronchial challenge test is the dose or concentration of the provocative agent that induces a 15% or a 20% decrease in the $FEV_1$ value ($PD_{15}FEV_1$, $PD_{20}FEV_1$ or $PC_{15}FEV_1$, $PC_{20}FEV_1$, respectively). The provocative dose (PD) represents a quantitative value of the provocative agent calculated by interpolation of the results of $FEV_1$ measurements after each increasing dose of the agent (Cockcroft *et al.* 1983b). The $PD_{15}$ $FEV_1$ value combines the information of bronchial hypersensitivity with the reactivity of ΔFEV1–15%. The raw data of a change in $FEV_1$ from the baseline (ΔFEV1) after each of the provocative dose level presents the hyperreactivity, however, only up to the maximun dose given at the test. These subjects, who do not reach a 15% decrease in $FEV_1$ with the maximum dose assessed in the protocol, are regarded as non-responsive (no BHR). (Hughes & Pride 2001, p.226)

Different BHR methods are listed in table 2, where constrictor delivery, method of inhalation technique, and the method specific cut off level for BHR are presented.

## 2.3. PATHOPHYSIOLOGY OF BHR

### 2.3.1 INTRODUCTION

Airway inflammation in asthma is complex and originates from a multi causal pathway in three different processes: acute inflammation, chronic inflammation and airway remodelling. Thus, several pathophysiological determinants are involved. (Barnes 2008, Diamant *et al.* 2010)

Increased ASM mass implicates in the pathogenesis of BHR and remodelling in patients with asthma (Borger *et al.* 2006, Hirst *et al.* 2004, Gosens *et al.* 2006, Johnson *et al.* 2001). ASM cell proliferation and hypertrophy, the pathways and mechanism, have been widely investigated during the past five years (Takeda *et al.* 2006, Tliba and Panettieri 2009). The mechanical changes in the bronchial tree and ventilation, which are typical for asthma's chronic inflammatory process, cause excess ASM contraction and BHR (An *et al.* 2007).

**Table 2.** Different BHR methods.

| **Method** cut off levels | **constrictor** | **nebulizer** | **inhalation techique** |
|---|---|---|---|
| **Chai *et al.* 1975** | Mch | Hudson neb 0.2 ± 0.02 ml/min flow rate 6L/min; 5 breaths FRC to TLC DeVilbiss42 neb attached to dosimeter | |
| **Cockcroft *et al.* 1977** | Hist | Wright neb, tidal breathing | |
| **Ryan *et al.* 1981** Hist $PC_{20}$≤8mg/ mL | Hist | DeVilbiss(Viasys) neb, Rosenthal-French dosimeter | |
| **Hargreave *et al.* 1981** | Mch | Wright neb 0.13 ± 0.015ml/min flow rate 6L/min, Mch 0.03–32.0 mg/ml 2 min tidal breathing | |
| **Yan *et al.* 1983** | Hist | hand operated technic; 3.1 mg/ml per inh (=0.03µmol), ad 3.9 µmol | |
| **Yan *et al.* 1983** | Hist | DeVilbiss646 neb, Rosenhal-French dosimeter; 0.06 s nebtime; 5 x 0.3 mg/ml (=0.006 µmol) ad 10 mg/ml | |
| **Nieminen 1992** Mch$PD_{20}$≤2.6mg | Mch | Spira2 jet neb dosimetric, inhalation synch. tidal breathing | |
| **Sovijärvi *et al.* 1993** Hist $PD_{15}$≤1.6mg | Hist | Spira2 jet neb dosimetric, inhalation synch. tidal breathing | |
| **Sterk *et al.* 1993** [ECSC _ERS Statement 1993] | Hist | DeVilbiss646 neb (0.13 ml/min), 2 min tidal breathing | |
| **Crapo *et al.* 2000** [ATS 1999] | | Sterk et al. 1993: 5-breath dosimetric method Cockcroft et al. 1977: 2 min tidal breathing | |
| ref. Cockcroft 2008 | Mch | DeVilbiss646 neb; dosimetric, 9µL per breath, 5 FRC to TLC [45µL aerosol at each concentration] | |
| ref. Cockcroft 2008 | Mch | Jett neb 0.13mL/min; 2 min modified tidal breathing [appr.90µL aerosol at each concentration] | |
| **Schulze *et al.* 2009** | Mch | APS by Viasys PD20 16mg/ml; normal Mch APS_SC >1mg | |

## 2.3.2. ROLE OF RESPIRATORY STRUCTURES, CELLS AND MEDIATORS IN BHR

### *2.3.2.1. Airway smooth muscle (ASM) and histamine*

Increased ASM in asthma, resulting from cell hyperplasia and hypertrophy, has been well recognized (Hirst *et al.* 2004, Munakata 2006). A number of factors affect ASM proliferation, such as growth factors, contractile agonists, extracellular matrix proteins, and other mediators, such as lysosomal hydrolase, tryptase, and cytokines (Panettieri 2008, p.89-104). These factors, in addition to oxidative stress, muscle stretch, a matrix upon which cells are grown, and inflammatory stimulus can trigger an increase in ASM proliferation *in vitro* (Hirst *et al.* 2004, Munakata, 2006). It has been challenging to prove these findings *in vivo*, however. Yick *et al.* (2012) have succeeded in demonstrating a structure-function relationship between extarcellular matrix in ASM and dynamics of airway function *in vivo*. The fractional area or density of ECM components in ASM between asthma patients and healthy controls with or without atopy showed no significant difference, but significant correlations between several EMC components and parameters reflecting bronchodilatation or bronchoconstiriction, such as metchacholine dose-response slope, existed among the asthmatics.

Airway smooth muscles exist around bronchi, and become more prominent in the smaller airways. ASM of the tertiary bronchus is typically spiral in shape, which makes it possible to contract both in length and diameter during expiration. The ASM plays a fundamental role in the bronchioles, which are less than 1mm in diameter, because no cartilage exists in the distal parts of the airway. The total crosssectional area of all bronchioles is greater than that of the rest of the conducting tract combined. The ASM tone is responsible for airflow resistance within the lungs. (Burkitt *et al.* 1993, p.226)

Smooth muscle is nonstriated and posesses a fusiform shape, actin, myosin, and intermediate filaments (desmin) form the contractile apparatus. Smooth muscles are rich in actin relative to myosin (12:1 in smooth muscle, 2:1 in skeletal muscle), and tropomyosin and troponin are not found in smooth muscles. Calmodulin functions as a regulatory protein in smooth muscle. (Michael & Sircar 2011, p.76-80)

Excitation of smooth muscle differs from that of skeletal muscle. The axon innervating smooth muscle forms multiple junctions throughout the muscle. Neurotranmitters, ACh or norepinephrine, are released from varicosities, and contraction occurs finally over a muscle membrane, as in the neuromuscular junction. Activation occurs also via diffuse junctions in single-unit smooth muscle, in which the neurotransmitter-containing varicoses do not contact with any single smooth muscle cell. Neurotransmitter is released in close proximity to the smooth muscles, which leads to an activation and a contraction. All muscle cells convert ATP biological energy into generation of force or shortening. (Michael & Sircar 2011, p.81-90)

### *ASM as part of the autonomic nervous system*

It is a conventional view that the ASM is primed to contract in response to neural stimulation or the release of inflammatory mediators in asthma (Hughes & Pride 2001, p. 222). However, there is a complex autonomic neural system, receptors, neurotransmitters, reflexes, and non-neural pathways that together conduct the orchestra of ASM contraction (Nadel 1980, p.217-257, An *et al.* 2007, Racke & Matthiesen 2004, Pavlinkova *et al.* 2003, Thurmond *et al.* 2008, Zampeli & Tiligada 2009).

Acethylcholine is the transmitter of the cholinergic synapses between the pre and post gangliolic neurons, and acts on nicotinic or muscarinic receptors on postganglionic neuron. There are five types of muscarinic cholinergic receptors (M1-M5), all coupled via G proteins to adenylate cyclase or phospholipase C. The M2 and M4 receptors are found in smooth muscle. Muscarinic receptors that act via open K and Na channels have methacholine as an agonist and atropine as an antagonist. The nicotine receptors act via K-channels only. (Ganong 1993, p.85-89 & 201-207)

The vagus nerve, which plays an important role in BHR, carries most of the parasympathetic preganlionic fibers, but also carries sensoryfibers and nerves to skeletal muscles. Parasympathetic afferent fibers bring information from the organs, and serve as reflex pathways. (Michael & Sircar 2011, p. 98-101)

The sympathetic system, which is also referred to as the adrenergic system, has three types of receptors on target organs: α1receptors mediate smooth muscle excitation, β2 receptors mediate inhibition of smooth muscle, and β1 mediate excitation of the cardiac muscle. The preganglionic transmitter is Ach, and the post-ganglionic transmitter is norepinephirine, which binds the strongest to α-receptors. As an exception, ACh exhibits neural excitation in the sweat glands and vasodilatation in the vessels of ASM. (Ganong 1993,p.85-89 & 201-207)

### *Activation of ASM in postganlionic receptors*

Activation of postsynaptic receptors causes an excitatory postsynaptic potential (EPSP), or in some cases an inhibitory potential. Binding of acetylcholine to nicotinic receptors leads to a fast EPSP (30ms), whereas the EPSP is slower (30s) through muscarinic receptors.

The cholinergic response leads to secretion of the nasopharyngeal and bronchial glands, and contraction of the bronchial ASM. Whereas noradrenergic impulses, both stimulation of the bronchial glands and ASM relaxation, are triggered via β-receptors. The inhibition of gland secretion only occurs via α-receptors (Michael & Sircar 2011, p.67).

To conclude, parasympathetic and sympathetic receptors in the bronchioli contribute to the actions of ASM: the cholinergic excitation leads to ASM contraction, and the adrenergic excitation of β2-receptors leads to ASM relaxation. (Michael & Sircar 2011, p.65-66)

### *ASM and afferent pathways from the epithelial respiratory wall*

The respiratory wall consists of sensory receptors, that mediate responses from physiological stimuli, such as pressure, tension, and temperature. These sensory receptors are nerve endings or specialized cells that convert stimuli from the environment, external or internal, into afferent nerve impulses, which pass in to the central nervous system, where they initiate voluntary or non-voluntary actions. The nerve endings are found in the supporting tissue, they are small in diameter, and typically slow in conduction rates. (Burkitt *et al.* 1993, p.132) Indirect stimuli, such as exercise or adenosine monophosphate (AMP), cause reactions via these neuronal pathways. Thus these stimuli are thought to be closer to the origins of the pathophysiology of asthma and/or spontaneous asthmatic reactions. (Hughes & Pride 2001, p.222).

The vagus nerve is supplied by afferent fibers that origin in the bronchial epithelium. As the sensory receptors, these afferents are stimulated by both physical and chemical factors. The resulting neural reflex causes broncoconstriction: foreign particles in the trachea trigger cough, as does sulfur dioxide ($SO_2$) and nitrogen dioxide ($NO_2$). The cold air, however, has a direct effect on the ASM and leads to a contraction. The neural reflex does not mediate this, as it acts in response to other physical or chemical factors. (Michael & Sircar 2011, p. 291)

### *Cell respiration, ASM contraction and BHR*

Respiration consists of two different but interrelated processes: cellular and mechanical respiration. Cell respiration is an energy process, in which high-energy substrates are derived from organic molecules via complex enzymatic processes. Mechanical respiration, in contrast, occurs in the respiratory system, and involves the gas exchange of inhaled and exhaled oxygen ($O_2$) and carbon dioxide ($CO_2$). (Michael & Sircar 2011, p.477-480)

Adenosine is a naturally occurring purine nucleoside. These two facts, cell respiration and adenosine, are linked together by adenosine metabolism (Polosa *et al.* 2002, van den Berge 2003). Asthmatics present elevated levels of adenosine in their respiratory airways (Driver *et al.* 1993). There is evidence that AMP-induced bronchoconstriction is mediated by activation of the $A_{2B}$ adenosine receptor (Feoktistov & Biaggioni 1995).

In optimal circumstances for energy generation, adenosine di-and triphosphates are converted to adenosine monophosphates, and further to high energy adenosine compounds (Michael & Sircar 2011, p.467). In situations such as hypoxemia, and in excessive cell stimulation, this process is interrupted. AMP is no longer converted to high-energy adenosine phosphates, but it is transported to the exterior of the cell and metabolised to adenosine (Polosa *et al.* 2002). Adenosine levels measured in bronchial lavage and in exhaled breath have been reported to be elevated among

asthmatics (Driver *et al.* 1993), similar to those measured after an allergen challenge (Mann *et al.* 1986).

### *Bronchoconstriction versus bronchodilatation*

The autonomic nervous system rules bronchoconstriction in the lower the airways. Parasympathetic, cholinergic fibers of the vagus maintain a basal contraction state of the ASM in the bronchi and bronchioles, which is called the bronchomotor tone.

The bronchodilation of ASM is more complex. The non-adrenergic and non-cholinergic (NANC) system is the principal driver of bronchodilatation. These NANC fibers reach the lungs via the vagus (Barnes *et al.* 1982). Wessler and Kirkpatrick (2008) have explained in detail of the non-neuronal cholinergic system in humans, in which they explained that dysfunction of the NANC system is involved in the pathogenesis of different diseases. Data exists of that mucosal inflammation is associated with increased Ach levels, thus interferes the normal actions of those non-neuronal cells that carry on components of the cholinergic system, such as epithelial cells, submucosal glands and smooth muscle fibers (Wessler & Kirkpatrick 2001). The antimuscarinic drugs that may be used for chronic airway diseases, thus antagonise both the neuronal and non-neuronal acetylcholine. But, acetylcholine is also active on the nicotinic receptors that are found on those mentioned non-neuronal cells, thus exhibiting Ach-dependent actions indirectly involved in mucosal inflammation.

Sympathetic adrenergic control is able to cause bronchodilatation. Beta-2 adrenoreceptors oscillate between two forms, activated and inactivated. The receptor is activated when it is associated with guanosine triphosphate (GTP). Following a replacement of GTP by guanosine diphosphate (GDP), the β2 receptor is returned to the inactivated, low-energy form. The β2-agonists act via binding to GTP, rather than inducing a conformational change in the receptor. (Johnson 2008, p.257)

Beta2-receptor activation is associated with an increase in intracellular cAMP, which is a result of stimulating the ATP to cAMP conversion, which is catalysed by adenylate cyclase. Furthermore, cAMP levels are also related to the activity of phosphoriesterase isoforms, which degrade cAMP to 5'-AMP. (Michael & Sircar 2011, p.477)

The mechanism responsible for induced ASM relaxation via cAMP is not fully understood. Protein kinase A activation leads to the phosphorylation of some proteins that regulate muscle tone (Johnson 2008, p.258). Cyclic AMP also triggers the inhibition of calcium ion release from intracellular sources, diminishing intracellular calcium stores, resulting in ASM relaxation. Inositol triphosphate IP3 production and phospolipase C levels have been measured lower in the absence of the β2 receptor, and higher in the presence of high β2 receptor density, which links these to the cross regulation of stimulatory and inhibitory cell responses. Recent evidence also suggests a cross-talk of inhibitory and excitatory pathways of ASM

relaxation via muscarine M2 receptors and β2 receptors, whereby M2 activation leads to attenuated cAMP accumulation (Johnson 2008, p. 258).

### *Histamine*

Histamine, a biogenic amine (Schnell *et al.* 2011), acts as a neurotransmitter and local mediator. It binds to histamine receptors in the respiratory epithelieum, consequently taking part in the inflammatory and immune responses, which are associated with ASM functions in a complex way. Overall, histamine acts on vascular smooth muscle cells, which leads to vasodilation, and its actions in endothelial cells increase vascular permeability (Thurmond *et al.* 2008).

Drugs, food, and allergens cause mast cell degranulation, which subsequently leads to histamine release. Four histamine receptors are known: H1, H2, H3, and H4 (Thurmond *et al.* 2008). Histamine exhibits its actions via G-protein coupled receptors, and the four receptors exhibit their actions in different ways. H3 receptors are presynaptic, and they inhibit the release of histamine and other transmitters via G protein. H1 receptor activates phospholipase C, and H2 receptors increase intracellular cyclic AMP. (Ganong 1993, p. 93) The role of the histamine H4 receptor is under vigorous investigation, because it is known to modulate eosinophilic migration. The H4 receptor has been regarded as a novel target for pharmacological modulation of histamine-mediated signals in immune reactions (Zampeli & Tiligada 2009).

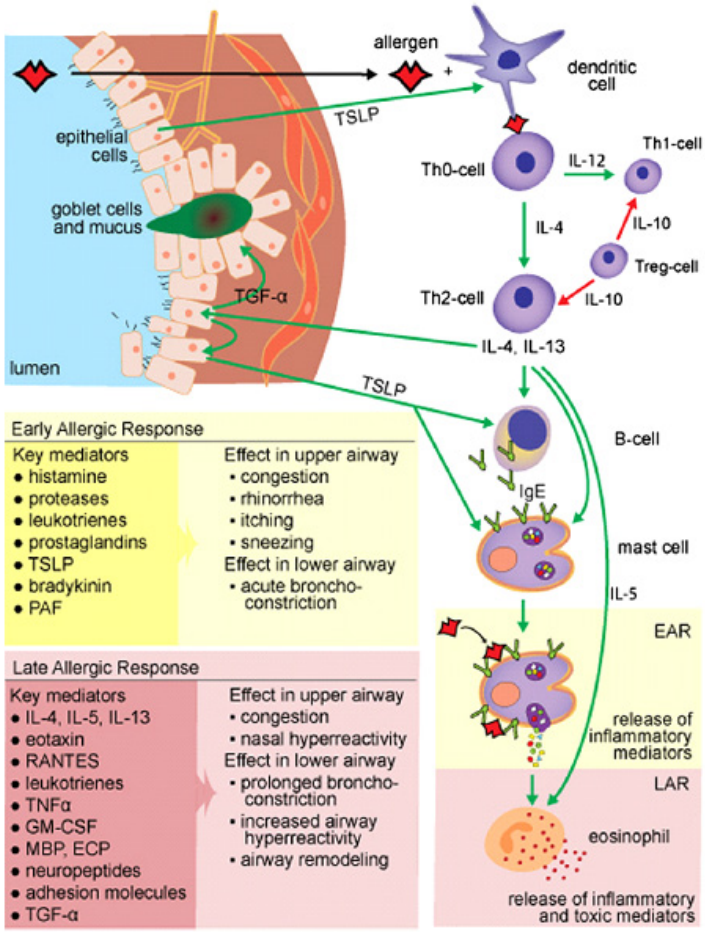
### *Histamine in action on ASM constriction*

For more than a century, histamine has been known to elicit local actions on the ASM, which lead to ASM constriction (Nadel 1980, p.229).

Histamine may be inhaled to trigger ASM constriction, exhibiting its effects via three different pathways in the airway epithelium (Nadel 1980, p. 231). First, it has local effects on the ASM . Second, histamine triggers reflex effects via receptors in the airways. Third, histamine affects the efferent parasympathetic pathways. Data from these studies in the assessment of histamine pathways are presented by Nadel (1980, p. 229). Researchers were also working on BHR prior to 1971, including such names as Dale, Laidlaw, DeKock, Sellick, Widdicombe, Hanh, Mills, and Karczewski (Nadel 1980, p.217-257).

### *Histamine reflex constriction*

It has been suggested that the reflex effect of histamine on bronchomotor tone is greater when histamine is delivered to the bronchial tree instead of the pulmonary artery (Nadel 1980, p. 222). These findings indicate that the receptors responsible for the constriction reflex are situated more proximally in the airways, in bronchi, rather than in the peripheral parts of the lower airways, i.e. in terminal bronchioles, alveolar ducts, or alveoli (Nadel 1980, p.222).




Reprinted with permission from Elsevier Diamant *et al.* 2010.

**Figure 1.**
The inflammatory cells and mediators of hypersensitivity reactions of the bronchial wall are presented.

Histamine has been reported to increase activity in the efferent vagal fibers to bronchi. It has been substantiated that for small doses of histamine, a vagal blockade abolishes its bronchial effects, as does atropine, and with higher dose of histamine, the histamine effects are diminished (Nadel 1980, p. 231).

Temperature has been suggested to effect the reflex pathways (Nadel 1980, p. 231). No histamine reflex reactions have been observed when cervical vagus nerves were cooled to 7-10°C. This was regarded as the range of temperature, in which the reflex response to histamine was abolished but the vagal efferent pathways were intact. (Nadel 1980, p. 231)

### *2.3.2.2. Inflammatory cells*

Diamant *et al.* (2010) has investigated cells and mediators that are involved in early (EAR) and late (LAR) allergic reaction, as presented in Figure 1.

Eosinophils in the tissues, blood, and bone marrow are increased in asthma (Kay 2005). Eosinophils have the potential to cause damage in the airway mucosa, but no firm evidence that eosinophils or their products directly cause BHR in clinical asthma exists (van Rensen *et al.* 2001, Kay 2005). A study by Leckie *et al.* (2000), using an interleukin (IL)-5 monoclonal antibody, did not support the role of eosinophils in causing BHR.

The major product of mast cells is tryptase, which has been reported to induce BHR, and to stimulate ASM responses via calcium signalling and cell proliferation (Berger *et al.* 2001). Mast cells are linked with ASM by released cytokines, such as IL-4, IL-13, or tumor necrosis factor (TNF)-α (Marthan *et al.* 2008, p. 129, 131, Holgate 2008)

The role of the monocyte-macrophage system in contributing to the pathology of asthma is less known (Holgate 2008). Macrophages are associated with phagocytosis, antigen presentation, and production of IL-1γ, IL-6, IL-12, and TNF-α. So called alternatively activated macrophages (AAMs) antagonize events of this classic interferon-γ pathway. AAMs respond to Th2 cytokines IL-4 and IL-13, which are associated with the development of chronic airway disease, i.e. mucus production and airway hyperresponsiveness. (Byers & Holtzman 2011)

TNF-α is strongly linked to the pathogenesis of asthma, as it acts as a proinflammatory cytokine (Holgate 2008; Marthan *et al.* 2008, p.131). It has been shown to induce BHR and sputum production (Thomas *et al.* 1995), and among asthmatics to enhance BHR after being administered by inhalation (Thomas and Heywood 2002).

#### *2.3.2.3. Respiratory epithelium*

Recent studies have shown that the respiratory epithelium does not act as a simple barrier of physical burden, but more as a regulator of the inflammatory and remodeling processes (Holgate *et al.* 2004, Holgate 2007, Gwilt *et al.* 2007, Slats *et al.* 2008, Mattila *et al.* 2011). Results of the large-scale, genome-wide association study of asthma (Moffatt *et al.* 2010) indicate that genes that interplay in the communication of epithelial damage to the adaptive immune system and activation of airway inflammation are associated with asthma.

Xiao *et al.* (2011) showed that epithelial tight junctions (TJs) are abnormal in asthma, thus creating a link between environmental exposure and airway vulnerability. Several impairments of the epitithelial barrier were found, such as formation of TJs, lower transepithelial electrical resistance (TER), and increased macromolecular permeability of the epithelium.

The NANC-system is centrally involved in the epithelial-cell inflammatory response. Acethylcholine (Ach) is pro-inflammatory for lymphocytes and epithelial

cells, but anti-inflammatory for mast cells and macrophages. For monocytes, Ach is both pro-and anti-inflammatory, and in neutrophils and esosinophils reactions are variable (Gwilt *et al.* 2007). The non-neuronal cholinergic system could be considered for targeted anti-muscarinic drugs which are applied to antagonize the effect of both neuronal and non-neuronal Ach as treatment of chronic airway diseases (Wessler & Kirkpatrick 2001 & 2008).

#### *2.3.2.4. Nitric oxide (NO) as a biomarker of airway inflammation*

Nitric oxide is a free radical that exhibits oxidative power in reacting rapidly with other molecules, such as oxygen and superoxide radicals (Török & Leuppi 2007). It has the potential to dilatate bronchial and vascular smooth muscle. Several cell types of the respiratory tract are capable of producing endogenous NO: endothelial cells of the epithelium and vessels, macrophages, eosinophils, and neutrophils. Bacteria of the saliva conduct the reduction of nitrate to nitrate, and further followed by a chemial reduction to NO (Marteus *et al.* 2005).

According to published data, an elevated exhaled NO concentration in asthmatics is beyond dispute (Alving *et al.* 1993, Kharitonov *et al.* 1994, Persson *et al.* 1994, ATS/ ERS 2005, ATS 2011). However, its association with direct and indirect bronchial challenges is more controversial (Leuppi *et al.* 2002, Spallarossa *et al.* 2003, Berkman *et al.* 2005), due to the different patterns of inflammatory cells involved in asthma (Laitinen & Laitinen 1994).

Exhaled NO has earlier been stated as the surrogate of eosinophilic bronchial inflammation typical for asthma, which has been restated (Alving & Malinovschi 2010). Eosinophila is basicly induced by IL-5, however, anti IL-5 treatment has no effect on asthma symptoms, lung function, or BHR (Leckie *et al.* 2000). A recent statement by Alving and Malinovschi (2010) suggests that exhaled NO serves as a marker of inhaled corticosteroid-response airway inflammation.

Cigarette smoking decreases the levels of exhaled NO by several mechanisms, both exogenous and endogenous (Török & Leuppi 2007). Tobacco smoke itself is rich in both reactive oxygen species (oxidants, superoxide anions) and NO. The reactive oxygen is believed react with the NO of the airways, which diminishes the measurable exhaled NO levels, and is also indicated to be the reason for the subsequent increasing levels of the reactions's end product, nitrate ($NO_3^-$), measured among smokers (Corradi *et al.* 1999 & 2003). Studies by others have demonstrated that the high cigarette smoke NO levels impact the endogenous production of NO via down-regulation of NO synthetase. Tobacco also damages NO-producing cells, thus interfering the NO-process (Maziak *et al.* 1998, Bhowmik *et al.* 2005, Kanazawa *et al.* 1996, Rengasamy & Johns 1993). In a longitudinal setting, the oxidant and

protease burden has been shown to retain in the airways, even symptoms improve after smoking cessation (Louhelainen *et al.* 2010).

### 2.3.3. TOBACCO SMOKE

Tobacco smoke contains over 4000 chemical particles, of which 250 are know to be unhealthy and over 60 compounds are defined as carcinogenic (http://www.cancer.org, http://www.stumppi.fi, http://www.suomenash.fi, Finland's ASH-Action on Smoking and Health, Helakorpi 2008)

Oxidative stress also contributes significantly to smoking introduced airway inflammation, related to changes in lung parenchyma and smaller airways (Langen *et al.* 2003). Rahman & MacNee (1998) have shown that oxidative stress is defined as reactive oxygen species (ROS) associated with BHR, and others have pointed out the association to mucus secretion and airway obstruction, in addition to activation of protease, and transcription of inflammatory genes (Kinnula *et al.* 1995).

Oxidative stress has been measured in an increased level of peroxynitrite in smokers and in COPD (Ito *et al.* 2004), and subsequently the cigarette smoke has also reduced the histone deacetylation (HDAC2) activity (Barnes 2009). Smoking leads to a decreased responsiveness in glucocorticoid treatment via decreasing the activity of histone deacetylation (HDAC2 ) (Ito *et al.* 2005): a persistant increase in the presence of activated chromatin exists, which associates with an increased transcription of inflammatory gene expression.

### 2.3.4. RESPIRATORY VIRUS INFECTION

Epidemiological asthma studies from early childhood have defined that severe episodic airway inflammation, which affects the rapidly growing lung and airway tissues, is strongly associated with the early initation of asthma (Holt *et al.* 2005, Stein 2008). Rhino virus (RV) (Gern *et al.* 2006) and respiratory syncytial virus (RSV) (Stein *et al.* 1999), which cause severe bronchiolitis in early childhood, have been defined as independent risk factors for early onset asthma when studied with atopy (Holt *et al.* 2010). Immunological networks in virus-induced immunopathology exist, where CD8+ effector T cells have been reported to be involved. Using an *in vivo* mouse model, Grayson *et al.* (2007) showed how the expression of the high-affinity IgE receptor on dendric cells links a viral infection to a chronic lung disease. Similarly, in a guinea pig model, Sutton *et al.* (2007) have reported that an induced RSV infection established persistent infection regardless of the host Th1/Th2 background, however, the host Th1 background limited the extent of the virus induced BHR and airway inflammation.

### 2.3.5. BROCHOMOTOR RESPONSES

#### *2.3.5.1. Airway smooth muscle*

ASM abnormalities, particularly associated with an increase in ASM, have been implicated in many studies (An *et al.* 2007). Sudden infant death syndrome (SIDS) is suggested to be caused by exaggerated ASM closure (Brown & Solway 2008, p. 61). Similarly, the ASM layer has been found to be thicker in asthmatics, compared to non-asthmatics (Carroll *et al.* 1993). Due to hyperplasia or hypertrophia, the increase of ASM should be associated with an increased contractile capacity, which has not yet been proven. Some studies have provided evidence against this theory (Okazawa *et al.* 1995), and suggested that the stiffness of the asthmatic airway wall is increased (Ward *et al.* 2001), and airway wall thickening might protect against excessive airway narrowing in patients with asthma (Niimi *et al.* 2003).

ASM tension has been proposed to be responsible for BHR (Brown & Solway 2008, p. 61). Recently, Tsurikisawa *et al.* (2010) demonstrated that ASM thickness is inversely correlated with BHR to histamine ($PC_{20}$, μg/mL), but not to acethylcholine ($PC_{20}$, μg/mL). These results further suggested that the BHR to histamine reflected airway remodelling, and particularly ASM hypertrophy.

In asthmatics, ASM cells present increased maximum shortening capacity and velocity compared to normal individuals. This has been suggested as an explanation for BHR and may be related to the phosphorylation state of the activation of the myosin light chain kinase, to that rate of actomyosin cross-bridge activity, and shortening velocity. (Brown & Solway 2008, p.62). The impaired relaxation of ASM has been proven in animal models of BHR (Brown & Solway 2008, p. 62). Barnes and Pride (1983) have shown that this relaxation is prolonged in sensitized airways, and also that the response to β-agonist was reduced.

#### *2.3.5.2. Heterogenity of ventilation and small pulmonary dimensions*

There has been relatively little published of the small pulmonary dimensions in comparison to the large body of research into the inflammatory associations of airway diseases in the assessment of BHR. However, BHR challenge testing, as a quantitative method to induce peripheral airway obstruction, has been recognized and commonly used in the assessment of small airway diseases (Burgel 2011). The small airways disease was the key topic of a European Research Seminar in 2010 which focused on the pathophysiology of the lung periphery in health and disease (Sterk & Bel 2011, Burgel 2011), thus stressing the need for a better understanding of the functional disturbances in the assessment of airway diseases and treatment (Scichilone *et al.* 2009, Ulrik & Lange 2011).

The heterogeneity of ventilation is a marked feature of bronchial constriction typical for peripheral airway disease, as demonstrated with an induced bronchoconstriction with methacholine (Bayat *et al.* 2009). It has been accepted that BHR testing plays an indisputable role in the development of new techniques for investigating pathophysiological events of the lung periphery (Niimi *et al.* 2000 & 2003, Gustafsson *et al.* 2003, King *et al.* 2004, Sterk 2004, Harris *et al.* 2006, Tgavalekos *et al.* 2007, Chapman *et al.* 2012). Two high-tech modalities of functional imaging, single-photon emission computed tomography (SPECT) (King *et al.* 2010), and positron emission tomography (PET) (Melo *et al.* 2010), have been introduced to clinical research of airway diseases as well. These diagnostic modalities represent a great potential for advances in coping with lung function dynamics, i.e. increasing the understanding of the patchy ventilation that is typical for small airway disease (King *et al.* 2010, Usmani & Barnes 2012).

## 2.4. METHODS TO ASSESS BHR

Standardisation of the protocols for the assessment of BHR have been requested for some time, in order to produce more comparable results between provocation agents and methods (Ryan *et al.* 1981, Beach *et al.* 1993, Jögi *et al.* 1999, Siersted *et al.* 2000). Comparison studies of the metacholine and histamine methods have been published before (Juniper *et al.* 1978, Juniper *et al.* 1981, Higgins *et al.* 1988). In some former studies, however, normal subjects have not reached the determined cut off point for brochial hyperreactivity, or healthy subjects have been excluded from the analysis (Chatham *et al.* 1982, Beach *et al.* 1993, Cockcroft & Berscheid 1983, Trigg *et al.* 1990). This causes difficulties in the interpretation of BHR results.

There is some discordance between the two ATS/ ERS 1999 recommended protocols for administration of methacholine (Crapo *et al.* 2000). The two-minutes tidal breathing method is considered "not comparable" to the dosimetric method where results of BHR are defined as the $PC_{20}$ and $PD_{20}$ values (Burke *et al.* 2009, Cockcroft *et al.* 2005, Prieto *et al.* 2006, Allen *et al.* 2005).

In early studies, the provocative agent has been delivered by continuously operating nebulizers (DeVilbiss, Wright) during deep tidal breathing (Cockcroft *et al.* 1977, Malo *et al.* 1983). This results in weakness in evaluation of the dose of the inhaled provocation agent (Nieminen *et al.* 1987, Beach *et al.* 1993), thus decreasing the level of accuracy, reproducibility, and functionality of the results. The application of the two-minutes tidal breathing method (Cockcroft *et al.* 1977) standardized some of the effects in inter- and intrasubjects variation, but has remained less reproducible in comparison to dosimetric tidal breathing methods (Chinn & Schouten 2005).

### 2.4.1. REPRODUCIBILITY AND SPECIFICITY OF BHR METHODS

Reports have documented reproducibility, sensitivity, and specificity of both the histamine and metacholine challenge tests (James & Ryan 1997), but predominantly among asthmatics. The reproducibility of challenge tests varies but is generally regarded as good (Juniper *et al.* 1978, Eiser *et al.* 1981, Dehaut *et al.* 1983, Balzano *et al.* 1989). By dosimetric tidal breathing methods, reproducibility has been improved (Nieminen *et al.* 1987 & 1988, Sovijärvi *et al.* 1993), the doubling doses being ± 0.72 and ± 0.65 (95 % CI), respectively. The specificity and sensitivity in asthma for the histamine challenge test, which has been employed in this study, has previously been reported to be remarkably high (for $PD_{15}FEV_1$ 1.0 mg 86% specificity and 92% sensitivity). For the cut off level histamine $PD_{15}FEV_1$ 0.4 mg, specificity was 100% and sensitivity 66% (Sovijärvi *et al.* 1993).

### 2.4.2. THE BHR METHODS USED IN FINLAND

In Finland, the bronchial reactivity response to methacholine is most usually measured by the Nieminen protocol (1992) and the response to histamine by the Sovijärvi *et al.* protocol (1993). The production of transition equations has been suggested, even for similar dosimetric methods, in order to be able to compare results (Jögi *et al.* 1999). At the commencement of this work, no transition equations were available for the present histamine and methacholine tests.

These direct, dosimetric, short methods of histamine and methacholine provocation, share a common origin of a longer, 10 steps program of the metacholine method, in which the highest cumulative dose of metacholine, 2.3 mg, was achieved by inhaling increasing doses and concentrations of methacholine (Nieminen *el at.* 1987). The histamine method used in the present study has has been evaluated to express very good day-to-day repeatability (Sovijärvi *et al.* 1993) and a high 0.95 intraclass correlation coefficient, as evaluated by Chinn and Schouten (2005).

Many of the lung function laboratories in Finland also provide so called “indirect challenge tests” (Anderson 2010), such as the free run test, inhalation of cold or dry air, hypercapnic challenge, or the mannitol test, in order to measure induced peripheral airway obstruction that is typical in asthmatic inflammation. The first two mentioned are in clinical use, the last mentioned used in research only. No comparison study of the direct and indirect methods used in the general adult population in Finland exist, either.

## 2.5. EPIDEMIOLOGY OF BHR, ASTHMA AND ALLERGY

### 2.5.1. PREVALANCE AND RISK FACTORS

Since the 1960s, research on asthma prevalence has demonstrated constantly increasing case numbers (Alanko 1970, Vesterinen *et al.* 1988, Woolcock 1991, Lundbäck *et al.* 1993, Peat *et al.* 1994, Kotaniemi *et al.* 2001, de Marco *et al.* 1998, Pallasaho *et al.* 2002, de Marco *et al.* 2012). Asthma was estimated to affect 24.6 million people in 2009 in the United States alone, where the current prevalence of asthma was 8.2% in adults. (Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/data/nhsr/nhsr032.pdf, *Accessed May 2012*).

In Helsinki, Finland, the self-reported prevalence of physician diagnosed asthma in the adult general population was 10%, based on a postal survey completed in 2006 (Kainu *et al.*, personal communication). This cross sectional study of the general population included 2449 individuals (age 20-69 years), and the study protocol was identical to that of a 1996 survey (Pallasaho *et al.* 1999). According to Kela -The Social Insurance Institution of Finland, which is the provider of social security benefits for all residents of Finland – reports (http://www.kela.fi/in/internet/english.nsf) of reimbursement of asthma medications in 2006, the concomitant prevalence of physicians' prescriptions for inhaled corticosteroids in the Hospital District of Helsinki and Uusimaa was 7.6%, in which inhabitants of all ages were included. The percentage for physician's prescriptions for any asthma medication was 26.7 %.

The most common and current opinion is that the prevalance and incidence of respiratory symptoms have started to decrease (Ekerljung *et al.* 2010, Anto *et al.* 2010, Lötvall *et al.* 2009), indicating a possible plateau of asthma prevalence. In contradiction, however, recent results of a cohort of young Italians, where data was collected in 2007-2010 and compared to data from 1998-2000, indicated that the prevalence of asthma is increasing after the decrement observed in in the 90s (de Marco *et al.* 2012). This may be due to an increasing proportion of untreated asthmatics nowadays in Italy (de Marco *et al.* 2012).

#### *2.5.1.1. BHR*

Patel *et al.* (2008) suggests that the increase in respiratory symptoms from the 1970s to the 1990s has levelled off in Western countries, but the percentages of wheezing symptom remain strickenly low in some developing countries, which indicates that great variation due to socio-geographic and environmental differencies exist probably in BHR as well.

Depending of the cohort and method for BHR measurement, data from 10 different population studies conducted in the years 1984-1992 points to a prevalence

**Table 3.** A summary of population studies on BHR.
Partly adopted from Jansen *et al.* 1997 and Grootendorst & Rabe 2004.

| Year | Age | no. subjects | BHR method<br>**Prevalence of BHR (%)** Asymptomatic BHR (%)<br>Prevalence of respiratory symptoms (%) |
|---|---|---|---|
| Weiss *et al.* 1984 | 6-24 | 213 | cold air; 9% ΔFEV1/VC<br>**22%** 11%<br>40% |
| Weiss *et al.* 1984 | A | 134 | cold air; 9% ΔFEV1/VC<br>1+1b<br>**16%** |
| Woolcock *et al.* 1987 | 18-88 | 916 | Hist; $PD_{20} \leq 3.9$ µmol or > 15% $\Delta FEV1_{n=12}$<br>**11%** 2%<br>56% |
| Rijcken *et al.* 1987 | 14-64 | 1905 | Hist; $PC_{10} \leq 16$ mg/ml<br>**25%** 14%<br>29% |
| Cockcroft *et al.* 1992 | 20-29 | 500 | Hist; $PC_{20} \leq 8$ mg/ml<br>**12%** 7%<br>10% |
| Sears *et al.* 1986 | 9 | 766 | MCh; $PC_{20} < 25$ mg/ml<br>**23%** 8%<br>27% |
| Salome *et al.* 1987 | 8-11 | 2363 | Hist; $PD_{20} \leq 7.8$ µmol<br>**18%** 7%<br>34% |
| Pattemore *et al.* 1990 | 7-10 | 2045 | Hist; $PD_{20} \leq 7.8$ µmol<br>**16%** 6%<br>33% |
| Toelle *et al.* 1992 | 7-12 | 210 | Hist; $PD_{20} \leq 7.8$ µmol<br>**13%** 7%<br>17% |
| Asher *et al.* 1988 | 6-11 | 2571 | Hist; $PD_{20} \leq 7.8$ µmol or > 20% $\Delta FEV1_{n=1}$<br>**18%** 9%<br>23% |
| Ernst *et al.* 2002 | p | 388 | MCh; $PD_{15} \leq 12$ µmol<br>**25-33%** |
| Riedler *et al.* 1998 | p | 613 | 4.5% hypertonic saline; $PD_{15} \leq 23$ml<br>**14%** |
| Peat *et al.* 1996 | p | 180 | Hist; $PD_{20} \leq 3.9$ µmol<br>**16.%** |
| Burney *et al.* 1987 | A | 511 | Hist; $PD_{20} \leq 8$ µmol<br>**14%** |
| Britton *et al.* 1994 | A | 2415 | MCh; $PD_{15} \leq 12.25$ µmol<br>**13%** |
| Xu *et al.* 1997 | A | 2684 | Hist; $PC_{10} \leq 8$ mg/ml<br>**18%** |
| Boulet *et al.* 1998 | A | 620 | Hist; $PC_{10} \leq 8$ mg/ml<br>**39%** |
| Leynart *et al.* 1997 | A | 799 | MCh; $PD_{20} \leq 4$ mg/ml<br>**34%** in women and **12%** in men |

Grootendorts & Rabe 2004: 1 g histamine= 3.26 mmol; 1g methacholine bromide =4.17 mmol.
P refers to a pediatric study cohort; A refers to an adult study cohort
Siersted *et al.* 2000: Mch $PC_{20} \leq 8$ mg/ml* correlates with Mch $PD_{20} \leq 0.5$ mg **
*$PC_{20}$ by Cockcroft *et al.* 1977, **$PD_{20}$ by Chinn *et al.* 1997

**Table 4.** Prevalence of BHR in selected populations.
Partly adapted from Grootendorts & Rabe 2004.

| Year | Age | no. subjects | BHR method<br>**Prevalence of BHR (%)** |
|---|---|---|---|
| *Healthy subjects* | | | |
| Sparrow *et al.* 1987 | A | 458 | MCh; $PD_{20}$ < 25 mg/ml<br>**30%** |
| Taylor *et al.* 1985 | A | 227 | Hist; $PC_{20}$ <16 mg/ml<br>**23%** |
| *Occupational* | | | |
| Frew *et al.* 1992 | A | 733 | MCh; $PC_{20} \leq 8$ mg/ml<br>**11%** |
| Storaas *et al.* 2007 | A | 197 | MCh; 2minutes' tidal, $PC_{20}$ ->slope<br>**21%** |
| *Asthma* | | | |
| Cockcroft *et al.* 1977 | A | 140 | Hist; $PC_{20} \leq 8$ mg/ml<br>**100%** |
| Sunyer *et al.* 1995 | A | 214 | MCh; $PC_{20} \leq 8$ mg/ml<br>**63%** |
| *COPD* | | | |
| Yan *et al.* 1985 | A | 57 | Hist; $PD_{20} \leq 3.9$ µmol<br>**46%** |
| Kanner *et al.* 1994 | A | 5 887 | MCh; $PD_{20} \leq 5$ mg/ml<br>**25%** in men and **48%** in women |
| Tashkin *et al.* 1992 | A | 5666 | MCh; $PD_{20} \leq 25$ mg/ml<br>**59%** in men and **85%** in women |
| *Skiers* | | | |
| Sue-Chu et al. 2010 | A | 58 | MCh; $PD_{20} \leq 1814$ µg<br>**40%**<br>AMP; $PD_{20} \leq 50.5$ mg<br>**8%**<br>Mannitol $PD_{15} \leq 635$ mg<br>**5%** |

A refers to an adult study cohort.

of BHR between 11 and 35% (Jansen *et al.* 1997, Jansen 1999). Grootendorst and Rabe (2004) summarized that the prevalence of BHR among adults is 10-16% and in children 16-30%, referring to Pattimore *et al.* 1990. A Swiss general population study, SAPALDIA, has described the prevalence of BHR to decrease from 17-12.5 % in the years 1991-2011, in which the proprtion of symptomatics of those 17% were 51% (Ackermann-Liebrich *et al.* 2005, Brutche *et al.* 2006, Curjuric *et al.* 2011). In the ERCS I studies, the BHR prevalences varied from 3 to 28% among the 16 participating countries, with a median prevalence of 13 % (Chinn *et al.* 1997).

As in many other investigations, results of multivariate regession analysis by Toelle *et al.* (2004) indicate that independent determinants for asthma symptoms in young adult life (at ages 23-25) are female gender (OR 1.7), having atopy (OR 2.6), BHR (OR 2.9), recent wheeze (2.1) and abnormal FEV1/ FVC ratio in childhood (in age 8-10 years) (OR 3.0). The risk factor profile is dependent on the cohort, thus more determinants for BHR in adulthood have been presented, such as smoking,

ETS, work related causes, obesity, or increase in weight, air pollution, rhinitis, FENO, and other physiological or ventilatory biomarkers (Hewitt 2008 , Chinn *et al.* 1997, Jansson *et al.* 2001 & 2006).

General population studies on BHR in the elderly are scarse, as seen from Table 3. Since overall life expectancy has increased, (http://www.who.int/gho/publications/world_health_statistics/EN_WHS2011_Full.pdf), such studies as presented by Scichilone *et al.* (2005), are of great value. This review included 18 BHR studies that had been conducted between 1983 and 2002. The results of this review suggested that BHR is increased by age, in which the prevalence of BHR appears to increase (Scichilone *et al.* 2005). The major determinants were reduced lung function, history of smoking, and atopy. The impact of female gender was suggested for further investigations. Another point for further investigation was the assessment of BHR among the elderly in defining whether the increased decline in $FEV_1$ represents the cause of BHR rather than being the consequence of it.

In a study that comprised 208 individuals from Central Manchester, aged 45-86 (Renwick & Connolly 1999), the increase in BHR was suggested to be an effect of baseline airway calibre. In that cohort, BHR was defined as methacholine $PD_{20} \leq 200$ μg (Renwick & Connolly 1999), and 34% of the subjects presented BHR.

Ten Brinke has reported (2001) BHR data for a hospital recruited cohort of 136 severe non-smoking asthma patients. The results indicated that air flow limitation is common in adult patients with severe asthma, and it is associated with adult onset asthma (OR 3.3; 95%CI 1.2-9.0), airway hyperresponsivenss to histamine, PC20 ≤ 1mg/ml versus > 1.0 mg/ml (OR 3.9; 95%CI 1.2-13.0), and sputum eosinophils ≥ 2% (OR 7.7;95%CI 2.4-25.1). In the cohort, a persistent air flow limitation was seen in 49% of subjects, in whom lung function parameters, the baseline $FEV_1$, $FEV_1$/ FVC and RV/ TLC of predicted, all three were significantly worse ($p< 0.001$) than found in the 70 other severe asthmatic patients without a persistent airflow limitation ten Brinke *et al.* 2001).

#### *2.5.1.2. Allergy*

Atopy could be defined as a positive result from a skin prick test (SPT) or a certain level of detected allergen specific IgE. The total serum IgE has commonly been used (ECRHS), although it is less sensitive, and is increased in non allergic conditions as well, such as smoking (Zetterström *et al.* 1981). The SPT results could be defined as a single SPT reaction ≥3 mm (Bousquet *et al.* 2012). Some studies have define an atopy index, in which they have calculated the ratio of positive reactions to all tested allergens, or a number of such positive SPT reactions, or a wheal sum, i.e. two times radius, of the positive reactions. Just one positive SPT result often is enough to define a person to be atopic or allergic.

Kerkhof *et al.* (2003) demonstrated that young adults with multiple sensitization had the highest risk for BHR defined as metacholine $PD_{20}$ ≤ 2mg/mL, whereas the prevalence of multisensitization in the older age group (45-70 years) was much lower, and did not associate with BHR. This study also demonstrated that the total serum IgE is associated with BHR only at older ages: in young (20-44 years), birch pollen and timothy grass were not associated to BHR, nor was the specific IgE for cat (< 3.5 kU/l), but in the older subjects, the specific IgE for cat (with levels 0.70-3.5 kU/l) increased the risk for BHR 7.5-fold (96%CI 1.7-32.6). Regardless of the age of the subjects, levels over 3.5 kU/ l of the specific IgE for house dust mite correlated with BHR in the young and old, but sensitization exclusively to timothy grass or pollen did not associate with BHR.

Boulet *et al.* (1997) published their study of 3371 patients with allergic symptoms, and grouped them into three categories according to allergic asthma, rhinitis, or both. The age groups for the analysis were defined as 0-5, 6-15, 16-25, 26-45, 46-65, and ≥ 66 years, and the proportions of the patients in these age groups were 2.5%, 27.0%, 21.6%, 34.2%, 12.6%, and 2.2%, respectively. Boulet *et al.* (1997) reported that the proportion of subjects sensitised exclusively to indoor allergens was 21.1%, and 5.8 % inclusively to outdoor allergens, and 53.2% exihibited allergy to both types of allergens. Indoor allergen sensitisation was strongly associated with asthma, while sensitisation to pollen only was associated with rhinitis.

Among the elderly subjects, SPT studies of the general population are scarse (Skassa-Brociek *et al.* 1987, Schichilone *et al.* 2011). Participants of the Korean Longitudinal Study on Health and Aging comprised 854 subjects, aged ≥ 65 years, among whom 33% presented an allergen induced wheal greater than 0 mm (Song *et al.* 2011). In this study, older age and female sex were associated with reduced skin reactivity. When evaluated among those who reacted, the wheal size did not decrease with age and no gender effect was observed. SPT reactivity was not associated with BMI, smoking, atopy, or reported allergic symptoms during the last 12 months, including wheezing or whistling in chest, sneezing or a runny nose, or blocked nose without cold. (Song *et al.* 2011)

Results from the longitudinal general population Vlagtwedde-Vlaardingen study of 1965 to 1990 indicated that smoking and blood eosinophilia (≥ 275 cells/ μL) in individuals increases the risk to respiratory symptom development, especially if eosinophilia occurs among individuals with hyperresponsivenss defined as histaminen $PC_{10}$ ≤ 8mg/ml (OR 3.67; 95% CI 1.75-7.67). Eosinophilia alone did not significantly associate with BHR. (Jansen 1999)

The prevalence of sensitization among individuals with physician diagnosed asthma shows variation: in the FinEsS-Helsinki study, the prevalence was 56%, whereas reports of ECRHS studies present higher numbers, from 63 to 81% (Pallasaho *et al.* 2006, Leynaert *et al.* 2012).

### 2.5.1.3. Rhinitis

Alleric rhinitis is an IgE mediated reaction on nasal mucosa, and eosinophils are considered the key effector cells (Kämpe *et al.* 2011). Most rhinitis patients do not develop asthma (Nielsen *et al.* 2009), even though BHR to histamine is heightened in many individuals with allergic rhinitis (Cockcroft *et al.* 1992, Skiepko *et al.* 2011).

Since the 1990s one of the major determinants for incident adult asthma has been rhinitis according to the reports of the ECRHS (Chinn *et al.* 2004, Torén *et al.* 2004). Thus, the Global Allergy and Asthma Network of Exellence ($GA^2LEN$) has developed a questionnaire in order to assess the presence of chronic rhinosinusitis (CRS) in a epidemiogical setting. In the very recent results, the $GA^2LEN$ survey (Jarvis *et al.* 2012) has reported the 2008-2009 prevalence of CRS among lifetime non-smokers in Europe to vary from 6.9% to 27.1%, in which the lowest prevalence is in Helsinki, Finland and Bradenburg, Germany, and the highest in Coimbra, Portugal (Jarvis *et al.* 2012). The average percentage of CRS was 10.9%. In the same report, the prevalence of asthma was 8.2%, and in Finland 7.8%, respectively. Rhinitis, which was defined as a yes answer to "Do you have any nasal allergies or hay fever?", was reported in all centers, on average by 30%, and in Finland by 40%, of the subjects.

Aronsson and colleges (2008) have reported that patients with allergic rhinitis and asthma symptoms manifested in greater peripheral airway obstruction compared to those with allergic rhinitis and BHR. The BHR method was based on a tidal volume triggered inhalation of methacholine with five increasing doses, where the maximun cumulative dose was 2000 μg. Methacholine /$FEV_1$ slope was used as the index of BHR. There was no diffrenece in the BHR slope between rhinitis with asthmatics or rhinitics with BHR, however slope for resistance in the frequency of 5 Hz, showed a significant increase from rhinitis patients, to rhinitis patients with BHR, and subsequently to patients with rhinitis and asthma ($p < 0.01$, in both respectively). These results indicate that rhinitis patients with asthma have a more peripherial obstruction than the rhinitis patients with or without BHR, thus also explaining their previous finding that asthmatics have a greater perception of bronchial obstruction (Aronsson *et al.* 2005).

Very recent results by Skiepko *et al.* (2011) revealed that in patients with seasonal rhinitis, the airway acid-base equilibrium and nitrogen metabolite concentration in EBC may serve as a new area for research. They found that that BHR associated with increased nitrite and fractional exhaled nitric oxide (FENO), and with decreased pH, which links with earlier observations of the conversion of endogenous nitrite to nitric oxide via acidification of the airways reported by Hunt *et al.* (2000). Thus, Skiepko *et al.* (2011) have hypothesized that measurement of the acid- base equilibrium and pH-dependent nitrite production leading to BHR would serve as a better diagnostic tool than the more commonly used FENO.

#### *2.5.1.4. Smoking*

Recent reports on Finnish smoking habits describe a decreasing trend in smoking in all age groups, both in men and women (Kinnula *et al.* 2011). The government policy of Finland has aimed to achieve a tobacco-free Finland by 2040, which means policies and regulatory actions in marketing and supply of tobacco products in retail sale facilities, especially targeted to teenage customers. These actions support the Finnish aim of a –10% annual decrement in daily smoking. (http://www.savutonsuomi.fi/en.php).

Exposure to environmental tobacco smoke has been regulated since 1995 in Finland when smoking in public places and at work become prohibited (Pietinalho *et al.* 2009). Although, four years after, of the non-smokers, 8% in men and 3 % in women reported that they were daily forced to work exposed to tobacco smoke, in which the figures for the smokers were 37% and 17%, respectively. (Helakorpi 2008, Helakorpi *et al.* 2008, http://www.savutonsuomi.fi/en.php).

In 2001, Janson *et al.* reported the effect of passive smoking on respiratory health and lung function. This European Community Respiratory Health Survey I (ECRHS) (Burney *et al.* 1994) was a cross sectional study of 36 centers in Europe, in which Finland was not represented. Between 1990 and 1994, the reported prevalance of passive smoking varied between 22% and 76%, being lowest in Uppsala Sweden (Blanc *et al.* 1999). Passive smoking was associated with BHR, which was not the case with other lung function measurements.

A 2006 follow up study by the ECRHS on smoking and passive smoking (Janson *et al.* 2006) indicated that active and passive smoking was reduced in all age groups for each 10 years followed, in all centers except in Tartu. The prevalence for quitting smoking was higher in men than women. Current smoking rates for the countries included in the 2006 report demonstrated a great variation in the smoking rates, in which the hightest rate for current smoking was reported in Albacete, Spain (47%), and the lowest in Uppsala, Sweden (14%).

Exposure to tobacco smoke has been associated with increased BHR in several studies (Janson *et al.* 2001, Chinn *et al.* 2005), and there have been similar findings for asthma and COPD (Grootendorst & Rabe 2004) In a study of healthy men, smokers displayed twice as high BHR rates as those who had never smoked (59% *versus* 23%) (Sparrow *et al.* 1987). (Table 4) In the setting of a longitudinal study, Hospers and colleges (2000) reported a correlation between increased mortality from COPD and the severity of hyperresponsiveness, and this also held true for those who had never smoked. In the assessment of risk factors for BHR, ETS is often considered as a confounder. However, it is difficult to quantify the magnitude of ETS (Hewitt 2008).

#### *2.5.1.5. Obesity*

During the past decades, both asthma and obesity have increased in prevalence worldwide (WHO consultation 2000, Boulet 2008). In obese women, the risk for asthma has been reported to be over two fold in comparison to the normal weight controls (Chen *et al.* 2005). The asthma incidence has been related to pre-existing obesity (Beuther & Sutherland 2007). The Nurse's Health Study, that comprised 85 911 women, concluded that the weight gain plays an important role in the incident asthma, also in respect to asthma severity (Camargo *et al.* 1999).

BHR studies in obesity are scarce, but increasing in number (Tantisera & Weiss 2001, Aaron 2008, Dixon *et al.* 2010). The recent results of the 11 years's follow up in the SAPALDIA study suggests that baseline BMI value is not associated with BHR, but the change in the reactivity slope is positively associated with BMI (Curjuric *et al.* 2011). The review by Shore (2010) summarizes 16 BHR studies on obesity in adults and in children. In four of the eight adult studies, a positive correlation existed, and in three studies no effect existed. Of the eight studies in children, five studies found a positive association in BHR with BMI.

### 2.5.2. INCIDENCE OF RESPIRATORY SYMPTOMS AND ASTHMA

Self-reported wheeze has fairly good specificity and sensitivity for BHR both in adults and children (Patel *et al.* 2008, Burney *et al.* 1989, Shaw *et al.* 1995). These two features of respiratory disorders often co-exist, although high wheezing rates might indirectly indicate undiagnosed asthma in some patients (Patel *et al.* 2008), and only a minority of those individuals with physician diagnosed asthma report wheezing (Court *et al.* 2002).

The role of BHR in context to asthma and respiratory symptoms might differ in between age groups (Kurukulaaratchy *et al.* 2003, Vonk *et al.* 2004, Riiser *et al.* 2012). The onset of BHR is uncommon during adolescence, but those 8-12 years-old children who have BHR carry an approximately four-fold higher risk for persistant wheezing (Xuan *et al.* 2002), and later for relapse or onset asthma in adulthood. Some children grow out of BHR (Riiser *et al.* 2012), and the severity of BHR commonly decreases through adolescence, as reported by the Environment and Childhood Asthma study from Oslo. In this Norwegian birth cohort study, only in 8% of the studied BHR increased from age 10 to 16 years.

Based on a 30-years follow up of asthmatic children (Vonk *et al.* 2004), 22% of the studied showed a complete remission in by the age of 32-42 years. Of those in clinical remission, however, 57% presented BHR and/or low function (Vonk *et al.* 2004). Curjuric *at al.* (2011) reported that in the Swiss general adult population study (SAPALDIA), BHR persisted in 47% of individuals aged 29-72 years, whereas 7% of the normoreactive individuals became hyperreactive during the 11-year follow up.

**Table 5.** The incident rates for asthma per 1000 persons year$^{-1}$ in men and women.

| | men | women | total | incidence per 1000 persons year$^{-1}$ |
|---|---|---|---|---|
| Huovinen *et al.* 1999 | | 1.5 | 1.7 | 1.6 |
| Huurre *et al.* 2004 | | | 2.1 | |
| Lundbäck *et al.* 2001* | 1.7 | 2.9 | 2.3 | |
| Torén *et al.* 2004 | 1.5 | 2.9 | 2.2 | |
| Aarhus | | | 1.6 | |
| Reykjavik | | | 3.6 | |
| Bergen | | | 3.1 | |
| Göteborg | | | 2.2 | |
| Umeå | | | 2.1 | |
| Uppsala | | | 2.1 | |
| Tartu | | | 0.5 | |
| Ekerljung *et al.* 2010* | 1.9 | 2.8 | 2.4 | |
| Anto *et al.* 2010 | | | 4.5 | |

*adjusted incident rates

#### *2.5.2.1. Incidence rates for asthma*

##### *Adjusted incident rates for asthma per 1000 person years*

Recent updates from Europen countries present that incident asthma has levelled off (European Lung White Book 2003, p.16-25), as is also found in other Western countries. Different labelling of obstructive diseases is common in different societies (Pallasaho *et al.* 2005), thus warranting the employment of some harmonized diagnostic criteria for comparison. (European Lung White Book 2003, GINA Guideline)

Rates for incident asthma are higher in women, as presented in Table 5. The rate for incidence of asthma is also reported higher when when older age groups are included in the cohort (Huurre *et al.* 2004). In respect to age trend analysis, these are in accordance with the other Finnish study (Huovinen *et al.* 1999), which reported a higher cumulative incidence over the 15 year's follow up for men (3.2%) and for women (4.0%) of the 50-60 years age-group in 1990 in comparison to the younger age-groups of 30-40 and 50-60 years.

#### *2.5.2.2. Determinants for incident asthma*

Atopy explains only a proportion of new-onset adult asthma (Leynaert *et al.* 2012) in comparison to pediatric asthma studies, in which a positive skin prick test is commonly reported as the main risk factor (OR 9.3) (Rönmark *et al.* 2001). More

than half of adult onset asthma cases have been recently reported to be non-allergic (Anto *et al.* 2010). Non-allergic asthma is understood to be poorly recognised, especially in women for whom more than 60% of new-onset asthma has been reported to be non-atopic (Leynaert *et al.* 2012).

The remission in childhood asthma is reported to be as high as 10% for a one-year follow-up period in 7-8 year old Swedish children, i.e. higher than the remission in adult onset asthma, in which the remission rate is much lower, from 5 to 15% during a ten-year follow-up period (Rönmark *et al.* 1999 & 2001 & 2007, Ekerljung *et al.* 2008). Family history of asthma is beyond dispute to be a constant determinant for incident asthma in all ages (Rönmark *et al.* 2001, Hedlund *et al.* 2006, Anto *et al.* 2010), thus heredity plays a significant role in the pathophysiological control of the onset of respiratory diseases (Prescott & Nowak-Wegrzyn 2011, Holt *et al.* 2005).

The outcome of childhood asthma at age 32-42 was studied by Vonk *et al.* (2004). Of those allergic asthmatic children (n=119), 30% were in clinical remission as adults BHR was the only sign of abnormality in 4% of the studied, where as BHR together with FEV1 ≤ 90% of predicted constituted 11%. On the other hand, of those individuals with persistent symptoms (15%), symptoms were the only sign of abnormality for 3%, but symptoms exhibited together with BHR and $FEV_1 \leq 90\%$ of predicted for 12%. In this study, BHR was defined as histamine $PC_{10} \leq 16$mg/ml, a value comparable with PC20 8mg/ml in the 2 minutes inhalation method with Hargreave (Vonk 2004).

In a Finnish birth cohort study (1967, n=2269) asthma incidence was evaluated from birth to young adulthood (Huurre *et al.* 2004). The results indicated that boys to have a higher incidence rate than girls, whereas from 17 to 22 years this was reversed, and in early adulthood (23-32 years) incidence rates for both sexes were equal, 2.1 /1000/ year. In the FinEsS-Stockholm study, the incidence rate for women was higher than in men, and in most of the adult studies this has been true for all age groups (Lundbäck *et al.* 2001, Torén *et al.* 2004). In the FinEsS-Stockholm study, however, the results of the univariate analysis for adjusted incident asthma and of the multivariate analyses no longer showed this gender effect.

Longitudinal data suggest that low socio-economic status associates with incident asthma (Beckett *et al.* 2001, Eagan *et al.* 2004, Hedlund *et al.* 2006, Ekerljung *et al.* 2010). After correction for age, gender, family history of asthma, smoking habits, and occupational exposure to dust gases or fumes, Hedlund *et al.* (2006) reported that the manual-worker industry was at risk for development of asthma by an OR 1.7 (95%CI 1.0-2.7). A work-related excess of asthma incidence was found in a Finnish study that included over 49 000 employee, aged 25-59 years during the follow up years of 1986-1998. It showed that over a hundred different occupations are associated with asthma, but typically in the majority such occupations in which different kinds of uncleanness exists (Karjalainen *et al.* 2002a). These results indicate

that asthma is evidently not a disease of middle and upper socio-economic classes, as may have been thought during the most popular years of the hygiene hypothesis.

The evidence from numerous epidemiological studies has convinced that rhinitis is one of the main risk factors for incident asthma (Håkansson *et al.* 2011), thus playing an important role in context to respiratory symptoms, asthma diagnosis and treatment strategies (ARIA, Allergic Rhinitis and its Impact on Asthma 2007. http://www.whiar.org/). In a large Swedish population-based case-control study (Environ**M**ent and **A**sthma in **P**-county, MAP-study) of 15 813 subjects included, showed that non-infectious rhinitis and smoking increased the risk for adult-onset asthma, especially among the non-atopic subjects (Torén *et al.* 2002).

BHR independently increased the risk for incident asthma and respiratory symptoms, as it is associated with an increased decline in the annual lung function (Brutsche *et al.* 2006, Boutet *et al.* 2007). Increased BHR is seen even in the absence of symptoms for bronchial asthma, especially during the pollen season (Skiepko *et al.* 2011), and this group of subjects have been suggested to have an increased risk for asthma later in life (Guerra *et al.* 2002). The causality and pathways from an upper airway disease to the development of a lower airway disease in certain individuals is not yet fully elucidated (Guerra *et al.* 2002, Bousquet *et al.* 2008). The ARIA (Allergic Rhinitis and its Impact on Asthma) and GA(2)LEN studies have put great effort into organising large-scale international studies to address these issues (Baena-Cagnani *et al.* 2008, Bousquet *et al.* 2009).

#### *2.5.2.3. Asymptomatic and symptomatic BHR*

The proportion of asymptomatic BHR in population studies has been reported to comprise from 2 to 14% of BHR cases, as reported by Jansen *et al.* (1997). In longitudinal asthma studies conducted in 1992-1994, BHR has been reported to preceed the incidence of respiratory symptoms or incidence of asthma (Peat *et al.* 1993), but opposite findings have also been suggested (de Gooijer *et al.* 1993).

Désirée Jansen from the Netherlands has published her thesis entitled "Symptomatic and asymptomatic airway hyperresponsiveness: epidemiological and pathological studies", in 1999. The results indicated that various parameters of lung function, such as a lower pre-challenge level of $FEV_1$ % predicted or $FEV_1$ % VC, a lower maximally attained level of $FEV_1$ % predicted between the ages of 20 and 25, and a faster preceding decline in $FEV_1$, were associated with AHR. Lower pre-challenge levels of lung functions, of % predicted, associated with the presence of respiratory symptoms, but only in subjects with AHR. (Jansen *et al.* 1999b)

# 3 AIMS OF THE STUDY

1. To study the agreement of methacholine and histamine methods in the assessment of BHR among subjects without physician diagnosed asthma or chronic bronchitis.
2. To define the prevalence of BHR in subjects without physician diagnosed asthma or chronic bronchitis in the general population in Kemi.
3. To determine the prevalence of BHR in the general adult population in Helsinki.
4. To assess the determinants and risk factors for BHR in the general population in Helsinki.
5. To investigate the association of smoking exposure with BHR severity.
6. To evaluate the determinants for BHR in terms of two cut off levels, BHRms and BHR, (histamine $PD_{15}$FEV1 ≤ 0.4 mg and BHR ≤ 1.6 mg, respectively).
7. To study the association of exhaled nitric oxide (FENO) with BHR.
8. To assess incidence of asthma and its risk factors in FinEsS-Helsinki by the replies of two postal surveys 11 year's apart.

# 4 MATERIAL AND METHODS

In the county of Lapland in northern Finland (Kemi), 79 subjects were included in a bronchial challenge study with methacholine and histamine in the years 1997-1998.

In Helsinki, 6062 subjects took part in a postal survey in 1996, and 292 of them participated in the bronchial challenge studies in 2001-2003. Later, 4302 subjects of those 6062 subjects were participating in a follow-up postal survey in 2007. A flow chart demonstrating the sampling for the cohorts studied in Helsinki are given in Figure 2.

The studies were approved by the ethics committee of Länsi-Pohja Central Hospital Ethics Committee in Kemi and the ethics committee of Helsinki University Hospital. All patients gave the written informed consent.

## 4.1. SUBJECTS AND STUDY DESIGN

### 4.1.1. SUBJECTS OF THE KEMI COHORT (STUDY I)

The FinEsS studies in North Finland (Kemi) started in 1995. The postal survey comprised 7937 subjects (age 20-69 years), and the participation rate was 84%. Of the responders of the southern part of the study area (n=3420), a random sample of 959 subjects were invited to clinical studies performed in 1995-1996. (Kotaniemi *et al.* 2001)

For Study I, 79 adult volunteers were included. All of them had taken part in the FinEsS postal questionnaire survey in North Finland in 1996 (Kotaniemi *et al.* 2001). The subjects were randomly selected from a group of the FinEsS participants who did not have asthma or chronic bronchitis and who were living in Kemi or its surroundings. They had answered "NO" to the following questions: 1)"Have you now, or have you had, any of the following diseases: a) asthma? b) chronic bronchitis or emphysema?"; 2) "Have you been diagnosed as having asthma by a physician?"; 3)"Have you been diagnosed as having chronic bronchitis or emphysema by a physician?". The age range of the studied subjects was 21-73 years (47% women). Their demographic data are presented in Table 6a.

### 4.1.2. SUBJECTS OF THE HELSINKI COHORTS (STUDIES III-IV AND II)

The flow chart of the cohorts of the FinEsS-Helsinki studies is presented in Figure 2.

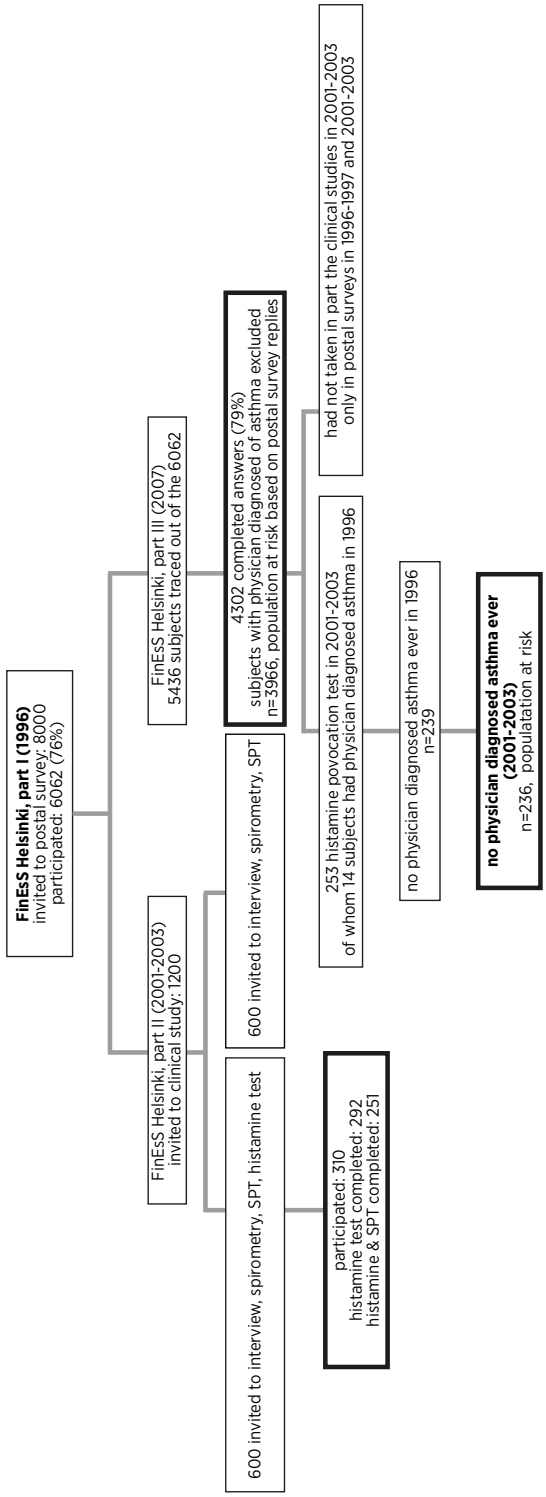



**Figure 2.** Flow chart for the study cohort in Helsinki.

**Table 6a.** Demographic data of the subjects studied in Kemi for BHR, n=79.

| | | Men (n=42) | Women (n=37) | Total (n=79) |
|---|---|---|---|---|
| | | mean±SD (range) | mean±SD (range) | mean±SD (range) |
| **Age (years)** | | 50.4 ± 15.7 (21–71) | 48.5 ± 12.7 (22-73) | 49.5 ± 14.3 (21-73) |
| **Height (m)** | | 174.3 ± 5.59 (1.61-1.86) | 162.9 ± 6.86 (1.46-1.74) | 1.69 ± 8.4 (1.46-1.86) |
| **Weight (kg)** | | 80.0 ± 12.6 (43-110) | 70.6 ± 13.8 (48-105) | 75.6 ± 14.0 (43-110) |
| **Body mass index (kg/m²)** | | 26.8 ± 3.1 (21.3-32.2) | 26.4 ± 5.2 (19.7-42.2) | 26.6 ± 4.2 (19.7-42.2) |
| **Spirometry** | FEV1 [L] | 3.40 ± 0.68 | 2.56 ± 0.48 | 3.01 ± 0.73 |
| | FEV1 of predicted [%] # | 96.5 (77.5-119.5) | 92.8 (77.4-118.5) | 94.8 (77.4-119.5) |
| | FVC [L] | 4.52 ± 0.95 | 3.31 ± 0.58 | 3.95 ± 1.00 |
| | FVC of predicted [%] # | 98.8 (77.3-122.6) | 97.4 (78.2-124.0) | 98.2 (77.3-124.0) |
| | FEV1/FVC [%] | 72.7 ± 7.4 | 74.0 ± 7.1 | 73.3 ± 7.2 |
| | FEV1/FVC of predicted [%] # | 97.5 (68.1-123.9) | 94.3 (75.8-116.7) | 96.0 (68.1-123.9) |
| | | n (%) | n (%) | n (%) |
| **Smoking history** | non-smokers n(%) | 16 (38.1) | 23 (62.2) | 39 (49.4) |
| | ex-smokers n(%) | 13 (31.0) | 3 (8.1) | 16 (20.3) |
| | smokers n(%) | 13 (31.0) | 11 (29.7) | 24 (30.4) |

Values are given as mean ± SD, and in values of the predicted [%] # as mean and range. Predicted values according to Viljanen *et al.* (1982).

### *4.1.2.1. BHR studies (Studies III–IV)*

The population for the FinEsS I postal survey (n=8000) was randomly selected from the Finnish population register (Väestökeskus) and designed to correspond to the general population living in Helsinki with respect to age and gender. The participation rate of the FinEsS I study was 76% (n=6062). Of the participants, 1200 were randomly invited to the FinEsS II clinical study, and half of those (n=600) were randomly selected to take part in the BHR study. The participation rate for the FinEsS II clinical study was 54% (n=643), and for the BHR study 45.4% (n=292).

The age-range of the studied subjects was 26-66 years. Their baseline $FEV_1$ varied between 60-136% of predicted (mean 94%) (Viljanen *et al.* 1982). Physician diagnosed asthma was reported by 6.2% (n=18) of the subjects, and the historical use of asthma medication was reported by 17.8% (n=52) and during the last 12 months by 7.5% (n=22). The demographic data are presented in Table 6b.

**Table 6b.** Demographic data of the subjects of the Helsinki cohort studied for BHR, n=292.

| | | Men (n=123) | Women (n=169) | Total (n=292) |
|---|---|---|---|---|
| | | mean±SD (range) | mean±SD (range) | mean±SD (range) |
| **Age (years)** | | 45.2 ± 9.5 (28-65) | 47.3 ± 10.6 (26-66) | 46.4 ± 10.2 (26-66) |
| **Height (m)** | | 1.74 ± 0.06 (1.61-1.86) | 1.63 ± 0.07 (1.46-1.74) | 1.69 ± 0.08 (1.46-1.86) |
| **Weight (kg)** | | 80.0 ± 12.6 (43-110) | 70.6 ± 13.8 (48-105) | 75.6 ± 14.0 (43-110) |
| **Spirometry** | FEV1 [L] | 4.06 ± 0.70 (2.35-5.90) | 2.87 ± 0.51 (1.71-4.50) | 3.37 ± 0.84 (1.71-5.90) |
| | FEV1 of predicted [%] # | 94 ± 12 (62-127) | 94 ± 12 (71-129) | 94 ± 12 (62-129) |
| | FVC [L] | 5.28 ± 0.82 (3.09-8.03) | 3.65 ± 0.61 (2.15-5.39) | 4.34 ± 1.07 (2.15-8.03) |
| | FVC of predicted [%] # | 99 ± 11 (67-127) | 99 ± 12 (72-145) | 99 ± 12 (67-145) |
| | FEV1/FVC [%] | 77 ± 6 | 78 ± 6 | 78 ± 6 |
| | FEV1/FVC of predicted [%] # | 95 ± 7 (71-113) | 95 ± 6 (80-115) | 95 ± 7 (71-115) |
| | MEF50 [L/ s] | 4.43 ± 1.33 (1.40-8.11) | 3.37 ± 0.98 (1.41-6.33) | 3.82 ± 1.26 (1.40-8.11) |
| | MEF50 of predicted [%] # | 82 ± 24 (30-147) | 77 ± 20 (39-137) | 79 ± 22 (30-147) |
| | Bronchodilatation from baseline, ΔFEV1 [%] | 3.1 ± 3.5 (-4-21) | 2.0 ± 2.9 (-5-15) | 2.4 ± 3.2 (-5-21) |
| **Exhaled nitric oxide (ppb) ¤** | | 20.48 ± 13.78 (2.43-74.60) | 16.37 ± 13.01 (2.13-95.60) | 18.09 ± 13.47 (2.13-95.60) |
| **Smoking** | pack years | 10.30 ± 12.57 (0-47) | 7.21 ± 10.18 (0-39) | 8.51 ± 11.33 (0-47) |
| | | n (%) | n (%) | n (%) |
| **Smoking history** | non-smokers n(%) | 47 (38.2) | 74 (43.8) | 121 (41.4) |
| | ex-smokers n(%) | 30 (24.4) | 45 (26.6) | 75 (25.7 ) |
| | smokers n(%) | 46 (37.4) | 50 (29.6) | 96 (32.9) |
| **ETS** | ETS ever§ | 84 (68.3) | 131 (77.5) | 215 (73.6) |
| | ETS both at work and at home | 49 (39.8) | 65 (38.5) | 114 (39.0) |

All values given as mean ±SD (range). Lung function values of the spirometry are also presented in values of the predicted [%], # as mean ±SD and range (n=291). Predicted values according to Viljanen *et al.* (1982). ¤ Exhaled nitric oxide (FENO), obtained from 277 subjects (men n=116, n=161 women), flow rate used 50ml/s. § at work and/ or at home.

**Table 6c.** Demographic data of the subjects in Helsinki participated in the postal survey in 2007, n=4302.

| | | Men (n=1745) | Women (n=2557) | Total (n=4302) |
|---|---|---|---|---|
| | | mean±SD (range) | mean±SD (range) | mean±SD (range) |
| **Age (years)** | | 53.5 ± 12.7 (31–80) | 53.1 ± 13.1 (31–80) | 53.4 ± 12.9 (31–80) |
| | | n (%) | n (%) | n (%) |
| **Smoking history** | non-smokers n(%) | 681 (39) | 1381 (54) | 2062 (48) |
| | ex-smokers n(%) | 593 (34) | 639 (25) | 1232 (29) |
| | smokers n(%) | 471 (27) | 537 (21) | 1008 (23) |
| **Physician diagnosed asthma** | | 136 (7.8) | 270 (10.6) | 406 (9.4) |
| **Asthma medication** | | 128 (7.3) | 292 (11.4) | 420 (9.8) |
| **Allergic rhinoconjunctivitis** | | 630 (36.1) | 1128 (44.1) | 1758 (40.9) |
| **Any wheeze** | | 340 (19.5) | 438 (17.1) | 778 (18.1) |
| **Shortnes of breath past 12 months** | | 226 (13.0) | 411 (16.1) | 637 (14.8) |

#### *4.1.2.1. Incidence of asthma (Study II)*

A follow-up study of the original FinEsS-Helsinki study cohort (n=6962) was performed in 2007 for the assessment of the incidence of asthma and respiratory symptoms. For this new postal survey (Appendix I and II) 5484 subjects were traced, and 4302 replies were obtained (participation rate 79%). Of the 578 subjects who were lost from the follow-up, 336 were dead, 8 were living abroad, 64 were traced without access to address, and 170 could not be traced. The demographic data of the cohort obtained is presented in Table 6c.

## 4.2. METHODS

### 4.2.1. LUNG FUNCTION MEASUREMENTS

#### *4.2.1.1. Spirometry in Kemi (Study I)*

The number of completed ventilatory function measurements was 683 (Kotaniemi *et al.* 2001&2005). Before the challenge tests a flow-volume spirometry with a Vmax22 Spirometer (SensorMedics Corporation, Yorba Linda, CA, USA) was performed according to ATS criteria (1994). A nose clip was used.

#### 4.2.1.2. Spirometry in Helsinki (Studies III-IV)

Altogether 643 acceptable baseline lung function measurements were performed during the FinEsS II clinical studies (Kainu 2008). In the BHR sub-cohort, which included 292 subjects, all except one had a valid baseline ventilatory function determined.

Subjects underwent flow-volume spirometry with a Vmax20c Spirometer (SensorMedics Corporation, Yorba Linda, CA, USA). The ATS 1994 standard was used for quality criteria for acceptable spirograms. Repeatability criteria followed the ERS1993 guidelines (Quanjer *et al.* 1993); the two largest FVC and $FEV_1$ were within 100 ml and 5%, and the two largest PEF within 10%. The calibration of the spirometer was performed with a 3-liter pump (syringe, Sensor Medics®, Sensor Medics Corporation) at least once a day. A nose clip was used, and a maximum of eight maneuvers were recorded.

The best three spirometric curves were chosen for analysis before and after the bronchodilation tests. The best curve was defined as the curve with the largest sum of $FEV_1$ and FVC values. The largest FVC and $FEV_1$, and the flow parameters from the best curve were recorded for the analyses. A trained technician performed all the spirometric measurements, and a physician interpreted the result for the study subject. Reference values of Viljanen *et al.* (1982) and European Community for Steel and Coal (ECSC, Quanjer *et al.* 1993) were used. The results were later re-computerized from the hard disk of the Vmax20c Sensor Medics.

#### 4.2.1.3. BHR testing in Kemi (Study I)

Bronchial challenge tests were performed in 382 FinEsS-Kemi subjects, of whom bronchial challenge tests with histamine and methacholine were administered to 86 subjects that were selected using a random procedure. Seven subjects were excluded from the analyses since their baseline FEV1 differed ≥ 10% on the BHR testing days. Finally, 79 subjects had valid bronchial provocation measured with both methods and were thus included in Study I.

The 79 subjects were tested for BHR with two different bronchial provocation methods (Nieminen 1992, Sovijärvi *et al.* 1993). Of them, 38 started with the histamine test and 41 with the methacholine test. In the latter group the mean interval between BHR tests was 7.8 (range 6-18) days, and in the former group 7.2 (range 6-10) days. The tests were performed between 8:20 AM and 3:30 PM.

Before the challenge tests, a flow-volume spirometry with a Vmax22 Spirometer (SensorMedics Corporation, Yorba Linda, CA, USA) was performed according to the criteria of ATS (1994). A nose clip was used. Inclusion criteria for BHR tests were as follows: a pre-test value of $FEV_1$ over 70% of predicted or over 2.0 L or the $FEV_1$/FVC ratio over 78% of predicted (Viljanen *et al.*, 1982), no respiratory

**Table 7.** The methacholine (Nieminen 1992) and histamine (Sovijärvi *et al.* 1993) challenge protocols.

| **Methacholine** | | | |
|---|---|---|---|
| phase | concentration (mg(ml) | no. inhalations | cumulative dose (µg) |
| 1 | 2,5 | 1 | 18 |
| 2 | 2,5 | 3 | 72 |
| 3 | 2,5 | 11 | 270 |
| 4 | 25 | 3 | 810 |
| 5 | 25 | 10 | 2600 |
| **Histamine** | | | |
| phase | concentration (mg(ml) | no. inhalations | non-cumulative dose (µg) |
| 1 | 4 | 1 | 25 |
| 2 | 16 | 1 | 100 |
| 3 | 16 | 4 | 400 |
| 4 | 16 | 16 | 1600 |

infection within 4 weeks prior to the tests, no severe heart diseases (myocardial infarction within 3 months, unstable coronary disease, dysfunction, arrhythmia) and no stroke. Subjects using asthma medication were excluded.

The methacholine test followed the protocol by Nieminen (1992). An inhalation-synchronized, dosimetric jet nebulizer (Spira Elektro 2, Respiratory Care Centre, Hämeenlinna, Finland) was used. Subjects breathed with a tidal volume of 0.5 ± 0.1 L, and the peak inspiratory flow was 0.5 L/s during the administration of aerosols. The nebulization of saline was followed by five increasing doses of methacholine chloride at 5-min intervals (0.018 mg; 0.072 mg; 0.270 mg; 0.810 mg; 2.600 mg as cumulative doses). (Table 7) $FEV_1$ was measured with the flow-volume spirometer. The end-point of the test was a decline of $FEV_1$ by 20% or more from the post-saline value or a completed protocol.

The histamine test was performed according to the method by Sovijärvi *et al.* (1993). A similar nebulizer was used as in the methacholine test. Subjects inhaled buffered histamine diphosphate aerosol according to the protocol with four doses (0.025 mg; 0.1 mg; 0.4 mg; 1.6 mg) at 5-min intervals (Table 7). $FEV_1$ was measured with the flow-volume spirometer. The end-point of the test was a decline of $FEV_1$ by 20% or more or a completed protocol.

During both challenge tests, all symptoms were recorded and lung sounds were listened to with a stethoscope. After the provocation, 400 µg of salbutamol aerosol was given via a spacer (Volumatic®, Glaxo Wellcome Production, Evreux, France). Post-bronchodilatation $FEV_1$ was measured after 10 min. The provocative doses inducing a decline of FEV1 of 15% and 20% ($PD_{15}FEV_1$; $PD_{20}FEV_1$ values) were

calculated for methacholine and histamine tests by interpolation (Cockcroft *et al.* 1983b). The duration of each challenge test was about 30 min.

The majority of the subjects did not achieve a 15% or 20% decline in $FEV_1$ at the highest dose of histamine or methacholine, respectively. Their PD values were considered censored, as it was only known that their PD was higher than the highest dose administered. The censored PD values were extrapolated using the one-point and two-point estimation methods (Cockcroft *et al.* 1977 & 1983b, Jokic *et al.* 1998) if the decline in $FEV_1$ was between 10% and 15% for histamine or between 13% and 20% for methacholine after the highest dose. For the final analysis, the one-point estimation method was chosen. The number of extrapolated PD values was 11 for the histamine test and 7 for the methacholine test. Regression analysis was applied to describe the possible linear association between the provocative doses (mg) of histamine ($PD_{15}FEV_1$) and methacholine ($PD_{20}$ $FEV_1$), including also extrapolated PD values when appropriate.

The classification of BHR severity used in this study, in terms of $PD_{15}FEV_1$, is based on an earlier clinical validation of the histamine challenge test (Sovijärvi *et al.* 1993): severe ≤ 0.100 mg, moderate 0.101-0.400 mg, mild 0.401-1.600 mg, and no BHR ≥ 1.601 mg. For the methacholine challenge, the limits were adapted from the distribution of $PD_{20}FEV_1$ methacholine in a population with asthmatic symptoms (Nieminen 1992, Hedman *et al.* 1998). In terms of $PD_{20}FEV_1$, the classes used were as follows: severe ≤ 0.150 mg, moderate 0.151-0.600 mg, mild 0.601-2.600 mg, and no BHR ≥ 2.601 mg. The dose response rate (DRR) to methacholine and histamine was calculated according to the formula: DRR = last FEV1 decline (%) / last dose administered (Peat *et al.* 1994).

#### *4.2.1.4. BHR testing in Helsinki (Studies III-IV)*

The BHR challenge test was carried out within 14 days from the clinical visit of the structured interview, spirometry, and skin prick tests (SPTs). The structured interview was done by a physician. A specially trained nurse performed the spirometry with the bronchodilatation test, and the SPTs (Pallasaho 2006), and the BHR challenge test with histamine.

Before the bronchial challenge tests a flow-volume spirometry with a Vmax22 Spirometer (SensoerMedics, USA) was performed according to the criteria of ATS (1994). A nose clip was used. The inclusion criteria for the BHR tests were: a pre-test value of $FEV_1$ over 60% of predicted (Viljanen *et al.* 1982) or over 1.5 L, no respiratory infection within four weeks prior to the tests, no marked heart diseases (myocardial infarction within 3 months, unstable coronary disease, dysfunction, arrhythmia), and no stroke. Subjects using asthma medication were not excluded.

The bronchial challenge was conducted with histamine by using a dosimetric tidal breathing method (Sovijärvi *et al.* 1993) in the same way as in Kemi. The end point of the BHR test was at least a 15% fall in $FEV_1$ or the used maximum non-cumulative dose of histamine 1.6 mg (Table 7).

#### *4.2.2.5. Fractional exhaled nitric oxide (FENO) measurement in Helsinki (Study IV)*

According to the FinEsS-II protocol FENO measurements were assigned for the same subjects as the BHR tests. Of the 600 randomly selected subjects, 310 participated of whom 295 presented technically acceptable FENO results. Earlier assessed by Rouhos (2010), the Helsinki cohort consisted of 73 healthy asymptomatic, nonsmoking subjects, in whom median and range of FENO was 14.0 ppb (2.1-49.8) (Rouhos *et al.* 2008). For the present study, 277 valid FENO measurements were obtained. The ATS (2011), Finnish (Rouhos *et al.* 2008) and Swedish guidelines (Olin *et al.* 2006) were used in assessment of clinically significant cut off levels, FENO ≥ 25 ppb, ≥ 30 ppb, ≥ 45 ppb, respectively.

All anthropometric data and values of the baseline ventilatory function for studies I, III and IV are presented in Tables 6a and 6b.

### 4.2.2. SKIN PRICK TESTS IN HELSINKI (STUDIES III-IV)

Skin prick tests (SPTs) were performed using the method recommended by a European Academy of Allergy and Clinical Immunology (EAACI) position paper (Johansson *et al.* 2001), except as single tests on one forearm. SPTs were done in the subjects who were younger than 61 years with 15 allergen extracts (cat, dog, cow, horse, birch, timothy, mugworth, *Alternaria alternata*, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Acarus siro*, *Lephidoglyphus destructor*, Cockroach, Latex) and 2 controls (positive control: histamine dihydrochloride 10 mg/ml; negative control: glyserin solvent).

Altogether 498 SPTs were performed in the clinical studies of FinEsS II in Helsinki (Pallasaho 2006). Among the 292 subjects who participated in the BHR studies, 271 SPSs were performed, whereas 251 valid SPTs were documented. The reason for excluded tests (7.4%) was a false positive reaction for the negative control solvent in 18 subjects (5 men, and 13 women) and a false negative for the positive control (histamine) in 2 subjects. In 239 subjects, valid SPTs, BHR, and FENO measurements were successfully completed.

### 4.2.3. POSTAL QUESTIONNAIRE (STUDIES I-II)

For both the original general population study cohorts in Lapland and Helsinki the same self-administered postal questionnaire was used. It was originally developed for the first Swedish OLIN survey (Lundbäck *et al.* 1991) in 1985 mainly from a revised version (Mikaelsson *et al.* 1982) of the British Medical Research council questionnaire (Medical Research Council's committee 1960), and further validated in the Swedish OLIN studies (Lundbäck *et al.* 2001, Lundbäck 1993). This postal survey has been used in the FinEsS studies in Sweden (Norrbotten, Örebro, Stockholm, and recently in Gothenburg), Estonia (Tallinn, Narva and Saarenmaa), and in Finland (Lapland and Helsinki) (Pallasaho *et al.* 2005).

For study II, which was a follow-up study performed in 2007 (FinEsS-Helsinki study of incidence), a self-administered postal questionnaire (Pallasaho *et al.* 1999) with two reminders was sent. The postal survey consisted of the same first 16 questions as the original postal questionnaire from 1996 with a few minor changes and, in addition, included six further questions due to local interests of the Helsinki part of the study. The extra questions were added to the original FinEsS-Finland questionnaire and were concerning smoking, health status, and living conditions during childhood. Further, questions related to cold climate, including working out-doors and exercise, were excluded.

#### *Definitions*

*Ever asthma* refers to the question "Have you ever had asthma?" and *physician-diagnosed asthma* to "Have you been diagnosed as having asthma by a doctor?"

*Allergic rhinoconjunctivitis* refers to "Have you had allergic eye or nose symptoms (hay fever)?"

*Asthma medication*: "Do you currently use asthma medicines (permanently or as needed)?"

*Any wheeze*: "Have you had wheezing or whistling in the chest at any time in the last 12 months?"

*Recurrent wheeze*: "Do you usually have wheezing or whistling in your chest when breathing?"

*Longstanding cough*: "Have you had longstanding cough during recent years?"

*Shortness of breath*: "Have you had asthma symptoms (intermittent breathlessness) or attacks of breathlessness?" with a further question about having had these symptoms during the previous year.

*Current smoking* refers to those currently or within one year having been smoking cigarettes, pipe or cigars.

*Ex-smokers* are those who quit smoking at least one year previously.

*Quitters* smoked in 1996, but were ex- or non-smokers in 2007.

*Starters* were ex- or non-smokers in 1996, but smokers in 2007.

*Countryside/farm living* refers to those who lived in the countryside/on a farm during the first 5 years of life.

*Family history of asthma* refers to those, whose parents, sisters or brothers have asthma.

*Family history of allergic rhinoconjunctivitis* refers to those, whose parents, sisters or brothers have allergic rhinoconjunctivitis.

*Crude cumulative incidence of asthma* was calculated by excluding from the population at risk those with either physician-diagnosed asthma or ever asthma in 1996, and calculating the proportion of new cases of physician-diagnosed asthma in 2007.

*Cumulative incidence of allergic rhinoconjunctivitis* was calculated by excluding from the population at risk those with the condition in 1996, and calculating new cases in 2007.

*Remission of asthma* refers to those with physician-diagnosed asthma and either use of asthma medication or symptoms of shortness of breath during the previous year or recurrent wheeze in 1996, but without these symptoms and medication in 2007.

*Remission of allergic rhinoconjunctivitis* refers to those who reported allergic rhinoconjunctivitis in 1996, but no longer in 2007.

### 4.2.4. CLINICAL INTERVIEW (STUDIES III-IV)

The clinical interview consisted of 162 questions about respiratory symptoms, family history of asthma and allergy, living conditions, occupation, smoking habits and exposure of environmental tobacco smoke. The complete interview form is presented in Finnish and in Swedish in Appendix III and IV. The questions have been referred in English in the thesis of Kainu (2008).

### 4.2.5. STATISTICAL METHODS

#### *4.2.5.1. Statistical methods in Kemi (Study I)*

The conventional crossover analysis, ANOVA for repeated measures, was used to compare the continuous variables of baseline $FEV_1$ between challenges. The McNemar test was applied to compare the dichotomous variables between histamine and methacholine tests. The Chi-square test was used to assess the effect of

demographic categorical variables on the BHR and agreement between challenges. DRR in histamine and methacholine tests was compared using the method of Altman and Bland (1983), and limits of agreement were calculated (Bland & Altman 1986) to indicate the level of agreement. Weighted kappa was applied in calculating the agreement of BHR severity between the two challenge methods (Liebetrau 1983). Agreement (%) and kappa coefficients were also assessed using different histamine and methacholine cut-off points with non-censored PD values. SPSS (version 12.0 for Windows, Chicago, USA) and StatXact 6 (Cytel Software Corp., MA, USA, 2003) were used for statistical analysis.

#### *4.2.5.1. Statistical methods in Helsinki (Studies II-IV)*

Age group, gender, and family history of allergic rhinoconjunctivitis served as covariates in calculation of risk factors for incident allergic rhinoconjunctivitis. Multiple logistic regression analysis was used because there was no information of time of events. The incidence rate was assumed to be linear and was calculated using the formula: $a/(11x(b-(a/2)))$ where $a$ is the incident cases and $b$ the subjects at risk in 1996. Adjusted cumulative incidence of asthma was calculated after excluding from the population at risk besides those with asthma, also subjects on asthma medication and those who reported physician-diagnosed chronic bronchitis or COPD in 1996. Further adjustment also excluded those reporting recurrent wheeze or shortness of breath during the previous year at baseline in 1996. Crude and adjusted incident rate ratios (IRR) by gender were calculated, and a p-value <0.05 and a 95% confidence interval were considered significant.

BHR severity, risk factors, and symptoms associated to BHR were determined at two different cut off levels of $PD_{15}FEV_1$. Risk factors for BHR were calculated by multiple logistic regression analysis including age, gender, family history of asthma. and determinants that were significant in the univariate analysis as independent variables. The results are expressed as odds ratios (OR) with 95% confidence intervals (CI). Chi-square test and Fisher's exact tests were used to assess differences between groups. P-value < 0.05 was considered statistically significant.

The programmes of Statistical Package for Social Sciences (SPSS version 15.0 for Windows, Chicago, IL, USA) and StatXact 8_2007(Cytel Inc., Cambridge, MA, USA) were used for the statistical analysis.

# 5 RESULTS

## 5.1. COMPARISON OF THE HISTAMINE AND METHACHOLINE METHODS (STUDY I)

Seventy-nine randomly selected subjects (21-73 years), without a diagnosis of asthma or chronic bronchitis, were examined. Fourteen percent of the subjects showed BHR (histamine $PD_{15}FEV_1$ ≤1.6 mg and methacholine $PD_{20}FEV_1$ ≤2.6 mg) with both of the methods, methacholine and histamine. Agreement between methacholine and histamine test results at different cut-off levels, defined as non-censored PD values, is presented in Table 8. The results of abnormal BHR agreed in 80% of cases (kappa =0.45; 95% CI 0.23-0.67), indicating moderate agreement. The agreement was good (96%, kappa 0.65), however, for moderate/ severe BHR ($BHR_{ms}$).

Good agreement was observed between the tests (weighted kappa 0.64; 95% CI 0.46-0.82) in assesment of BHR severity. Classification of BHR severity according to PD values in the methacholine and histamine tests is presented earlier on page 51. $BHR_{ms}$ was found in 6.3% of subjects (n=5) with the histamine challenge and in 5.1% (n=4) with the methacholine challenge, while mild BHR appeared in 21.5% (n=17) and 15.2% (n=12), respectively.

A linear association between histamine $PD_{15}FEV_1$ and methacholine $PD_{20}FEV_1$ was estimated, including extrapolated PD values in the analysis. The regression line was $PD20 = 1.088 + 0.967 \times PD15$ ($R^2$=0.17, n=19). No significant difference was present in DRR values obtained from the histamine (mean -8.40 [%/mg]) and methacholine (mean -7.97 [%/mg]) challenges (p=0.751).

In subjects with normal spirometry ($FEV_1$, $FEV_1$/FVC, PEF, $MEF_{50}$) at baseline, the agreement of positive BHR in the histamine and methacholine challenges was 86%. Decreased baseline FEV1 (<80% of predicted) did not significantly affect the agreement (81% vs. 67%, p=0.407). Neither smoking history (p=0.468) nor body mass index (BMI) had any significant impact (p=0.455).

We found better agreement between $PD_{15}$ histamine (< 1.6 mg) and $PD_{15}$ methacholine (< 2.6 mg) than with $PD_{15}$ histamine (< 1.6 mg) and $PD_{20}$ methacholine (< 2.6 mg), the kappa values being 0.64 and 0.45, respectively (Table 8).

The histamine test induced some respiratory symptoms, such as loss of voice, throat irritation, cough, and dyspnoea in 63% of subjects (n=50). During the methacholine test, the incidence of symptoms was slightly lower; 47% of subjects (n=37) reported one or more respiratory symptoms (p=0.047). No difference was present between the tests concerning the appearance of adventitious lung sounds by

**Table 8.** Agreement between histamine and methacholine challenges using different non-censored PD (provocative dose) cut-off points.

| Histamine ($PD_{15}FEV_1$) | Methacholine | Agreement % | Kappa (95% CI) |
|---|---|---|---|
| ≤ 0.4 mg | $PD_{20}FEV_1$ ≤ 0.6 mg | 96 | 0.65 (0.28-1.02) |
| ≤ 1.0 mg | $PD_{15}FEV_1$ ≤ 1.3 mg | 87 | 0.61 (0.39-0.83) |
| ≤ 1.0 mg | $PD_{15}FEV_1$ ≤ 1.4 mg | 86 | 0.58 (0.36-0.80) |
| ≤ 1.0 mg | $PD_{15}FEV_1$ ≤ 1.7 mg | 85 | 0.55 (0.33-0.78) |
| ≤ 1.0 mg | $PD_{20}FEV_1$ ≤ 1.3 mg | 87 | 0.61 (0.39-0.83) |
| ≤ 1.0 mg | $PD_{20}FEV_1$ ≤ 1.4mg | 82 | 0.37 (0.11-0.62) |
| ≤ 1.0 mg | $PD_{20}FEV_1$ ≤ 1.7 mg | 86 | 0.54 (0.30-0.78) |
| ≤ 1.6 mg | $PD_{15}FEV_1$ ≤ 2.6 mg | 86 | 0.64 (0.44-0.83) |
| ≤ 1.6 mg | $PD_{20}FEV_1$ ≤ 2.6 mg | 80 | 0.45 (0.23-0.68) |

lung auscultation; 9% of subjects showed either expiratory or inspiratory wheezing sounds during both tests.

## 5.2. PREVALENCE OF BRONCHIAL HYPERRESPONSIVENESS IN FINLAND (STUDIES I, III-IV)

### 5.2.1. PREVALANCE OF BHR IN KEMI (STUDY I)

Seventy-nine randomly selected subjects (21-73 years) without a diagnosis of asthma or chronic bronchitis were examined. The proportion of subjects with abnormal BHR (responders) assessed by the histamine test ($PD_{15}FEV1$ ≤ 1.6 mg) was 28%, and by the methacholine test ($PD_{20}FEV1$ ≤ 2.6 mg) was 20%.

### 5.2.2. THE PREVALENCE OF BHR, RESPIRATORY SYMPTOMS, AND DEREASED LUNG FUNCTION IN HELSINKI (STUDIES III-IV)

In Helsinki, 292 subjects of a random sample of the general adult population were examined. The proportion of subjects with BHR measured by the histamine test ($PD_{15}FEV_1$ ≤ 1.6 mg) was 21.2% (n=62). $BHR_{ms}$ ($PD_{15}FEV_1$ ≤ 0.4 mg) was found in 6.2% (n=18).

Of the studied subjects, 11% had $FEV_1$ below the lower limit of normal (LLN) (<80% of predicted), 13% had the ratio of $FEV_1$/FVC below LLN (≤ 88% of predicted) (Viljanen *et al.* 1982). FENO was 20.5 ppb±13.8 (mean ± SE) in men, and 16.4± 13.0 ppb in women. Of those studied, 19% (n=52) presented FENO ≥ 25 ppb, whereas

**Table 9.** Allergic sensitization, respiratory symptoms, and asthma, n=292.

| | Men | Women | Total |
|---|---|---|---|
| | n=123 | n=169 | n=292 |
| 1 positive SPT* reaction, n (%) | 55 (50.0) | 63 (44.7) | 118 (47.0) |
| ≥ 6 positive SPT* reactions, n (%) | 10 (9.1) | 5 (3.5) | 15 (6.0) |
| Allergic rhinoconjunctivitis (ARC), n (%) | 40 (32.5) | 71 (42.0) | 111 (38.0) |
| Family history of asthma, n (%) | 17 (13.8 ) | 36 (21.3) | 53 (18.2) |
| Physician-diagnosed asthma, n (%) | 7 (5.7) | 6 (3.6) | 13 (4.5) |
| Asthma medication ever, n (%) | 21 (17.1) | 31 (18.3) | 52 (17.8) |
| Asthma medication past 12 months, n (%) | 9 (7.3) | 13 (7.7) | 22 (7.5) |
| Inhaled corticosteroids§ | 3 (2.4%) | 6 (3.6%) | 9 (3.1%) |

Figures indicate numbers of subjects and their percentage in the groups.
*SPT: skin prick test were performed for men (n=110) and women (n=141) < 61 years (n=251).
§daily use of inhaled corticosteroids ≥ 200 µg

5.8% (n=16) had FENO ≥45 ppb, of whom 38% (n=6) had BHR. Prevalence of respiratory symptoms and diseases is included in Table 9.

BHR demonstrated no association with age, gender, BMI, and family history of asthma. The prevalence of mild BHR was higher among women than men (18.9% vs. 7.1%; p=0.010), however, and $BHR_{ms}$ associated significantly with aging (p=0.028). The mean age of subjects having $BHR_{ms}$ was higher compared to subjects having histamine $PD_{15}FEV1 > 0.4$ mg (51.5 vs. 45.8 years, p=0.049). The prevalence of individuals with asymptomatic BHR was 2.7%. Among those who had BHR, 12.3% were asymptomatic.

The distribution of BHR severity in subjects without physician-diagnosed asthma or chronic bronchitis in the general population was similar in Kemi and in Helsinki (Figure 3, p=0.241). In addition, in a sub-analysis, the proportion of BHR, or $BHR_{ms}$ only, did not show a significant difference between the subjects in Kemi and in Helsinki, p=0.158 and p=0.961, respectively.

BHR defined as $PD_{15}FEV1 \leq 1.6$ mg among those who reported shortness of breath (SOB) and wheezing during the past 12 months or reported SOB and nocturnal asthma symptoms/wheezing was found in 76.5%. Reported SOB or wheezing due to any food (61.5%), wheezing with breathlessness without having a cold (triad 52%), use of asthma medication during past 12 months (50%), were more common among those with BHR than in the rest of the study cohort (p=0.001, p<0.001 and p=0.002, respectively).

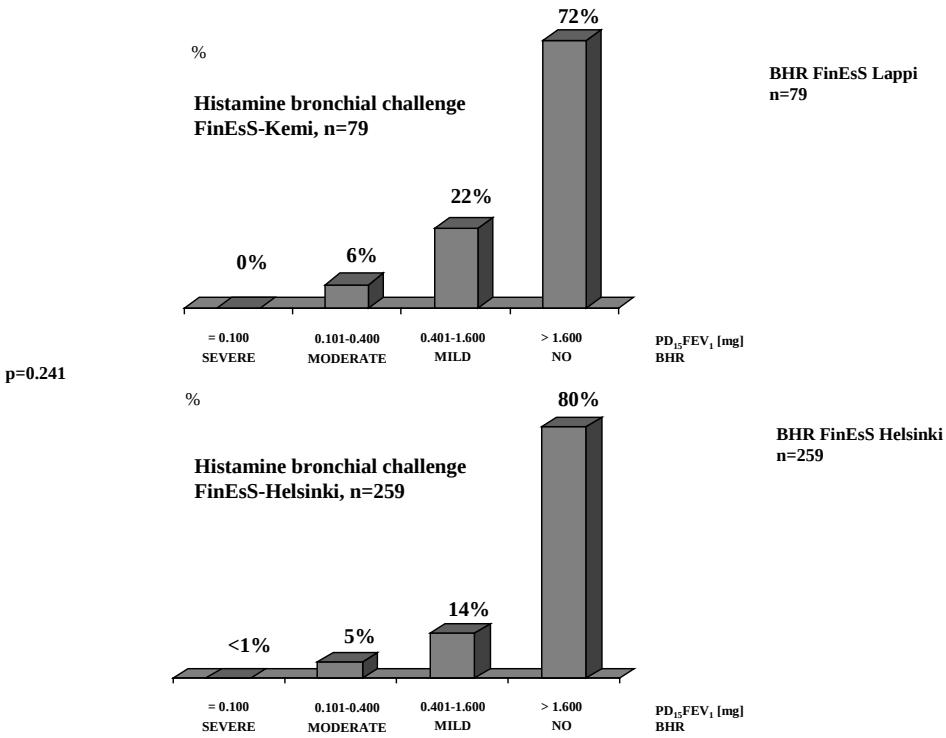



**Figure 3.** Prevalence of different levels of BHR among subjects with no physician diagnosed asthma or chronic bronchitis in the general adult population of FinEsS-Kemi and FinEsS-Helsinki cohorts.

Among those with $BHR_{ms}$, 83.3% (n=15) were smokers or ex-smokers, 77.8% (n=14) had been exposed to environmental tobacco smoke both at home and at work, 55.6% (n=10) had baseline $FEV_1$ below the normal range (Viljanen *et al.* 1982).

Sensitization to at least 6 allergens was significantly associated with $BHR_{ms}$ (p=0.018). Impaired lung function ($FEV_1$ < 80% of predicted), ever wheeze, nocturnal asthma symptoms, SOB, and wheezing during the past 12 months increased the risk for $BHR_{ms}$ remarkably, as presented in Table 10. Three of the subjects (16.7%) had physician-diagnosed asthma (*versus* $PD_{15}FEV_1$ >1.6 mg 8.1%, p=0.284). Compared to the original study population there was no significant difference in the proportions of subjects having BMI > 30 (16.7% *versus* 16.4%) or of subjects being sensitized for at least one tested allergen (46.2% *versus* 47.0%, p=0.438). The age group of 47-56 years (n=87, 30% of the studied) presented a diminished risk for $BHR_{ms}$ (p=0.043; OR 0.111, 95% CI 0.013-0.933).

**Table 10.** Risk factors, demographic variables, and data for asthma, respiratory symptoms and lung function of the study cohort (n=292).

| | | PD15≤1.6 mg, n=62 | | PD15≤0.4 mg, n=18 | |
|---|---|---|---|---|---|
| | **no. (% of 292)** | **N (% of no.)** | **OR (95%CI)** | **N (% of no.)** | **OR (95%CI)** |
| **Age** | | | | | |
| 26 < 41 years | 100 (34.2) | 20 (20.0) | 1 | 5 (5.0) | 1 |
| 41 < 53 years | 96 (32.9) | 18 (18.8) | 0.92 (0.45-1.88) | 2 (2.1) | 0.40 (0.08-2.14) |
| 53 - 66 years | 96 (32.9) | 24 (25.0) | 1.33 (0.68-2.62) | 11 (11.5) | 2.46 (0.82-7.36) |
| **Gender** | | | | | |
| men | 123 (42.1) | 20 (16.3) | 1 | 8 (6.5) | 1 |
| women | 169 (57.9) | 42 (24.9) | 1.70 (0.94-3.08) | 10 (5.9) | 0.90 (0.35-2.36) |
| **BMI** | | | | | |
| > 30 | 48 (16.4) | 12 (25.0) | 1.29 (0.63-2.67) | 3 (6.3) | 1.02 (0.28-3.66) |
| **Ventilatory function #** | | | | | |
| FEV1 < 80 % of pred | 32 (11.0) | 17 (53.1) | **5.39 (2.51-11.58)** | 10 (31.3) | **14.26 (5.11-39.82)** |
| FVC < 80% of pred | 12 (4.1) | 5 (41.7) | 2.78 (0.85-9.09) | 0 | 0 |
| MEF50 < 63% of pred | 77 (26.4) | 37 (48.1) | **6.99 (3.79-12.89)** | 15 (19.5) | **17.02 (4.77-60.68)** |
| FEV1/FVC < 88% of pred | 37 (12.7) | 18 (48.6) | **4.52 (2.20-9.31)** | 9 (24.3) | **8.75 (3.21-23.86)** |
| FEV1< 80% of pred & FEV1/FVC < 88% of pred | 11 (3.8) | 7 (63.6) | **7.16 (2.02-25.32)** | 7 (63.6) | **42.80 (10.89-168.15)** |
| FEV1 < 80% & MEF50 < 63% of pred | 24 (8.2) | 15 (62.5) | **7.80 (3.22-18.89)** | 10 (41.7) | **23.13 (7.90-67.69)** |
| Reversibility in FEV1 (ΔFEV1 [L]+12% and ≥ 0.2 L) | 4 (1.4) | 3 (75.0) | **11.64 (1.19-113.98)** | 1 (25.0) | 5.31 (0.52-53.83) |
| **Family history of asthma** | 53 (18.2) | 14 (26.4) | 1.43 (0.72-2.84) | 4 (7.5) | 1.31 (0.41-4.16) |
| **BHR tested in April-June** | 83 (28.4) | 19 (22.9) | 1.15 (0.62-2.11) | 9 (10.8) | **2.70 (1.03-7.07)** |
| Multisensitization (SPT ≥ 6 allergens)* | 15 (6.0) | 4 (26.7) | 1.42 (0.43-4.67) | 3 (20.0) | **4.67 (1.16-18.78)** |
| **Severe resp. infection at age < 5 years** | 46 (15.8) | 16 (34.8) | **2.32 (1.17-4.61)** | 4 (8.7) | 1.58 (0.50-5.03) |
| **Physician-diagnosed asthma ever** | 13 (4.5) | 5 (38.5) | 2.43 (0.77-7.23) | 3 (23.1) | **5.28 (1.31-21.22)** |
| **Asthma medication ever** | 52 (17.8) | 17 (32.7) | **2.11 (1.08-4.09)** | 6 (11.5) | 2.48 (0.89-6.94) |
| **Asthma medication (past 12 months)** | 22 (7.5) | 11 (50.0) | **4.29 (1.76-10.45)** | 4 (18.2) | **4.06 (1.21-13.62)** |
| **Symptoms** | | | | | |
| Ever wheezing | 134 (45.9) | 41 (30.6) | **2.88 (1.60-5.18)** | 16 (11.9) | **10.58 (2.39-46.90)** |
| Shortness of breath past 12 months | 60 (20.5) | 23 (38.3) | **3.08 (1.65-5.74)** | 8 (13.3) | **3.42 (1.29-9.08)** |
| Shortnss of breath & wheezing past 12 months | 17 (5.8) | 13 (76.5) | **14.99 (4.69-47.93)** | 4 (23.5) | **5.74 (1.66-19.88)** |
| Shortness of breath & wheezing at night | 15 (5.1) | 9 (60.0) | **6.34 (2.16-18.58)** | 4 (26.7) | **6.83 (1.93-24.19)** |

Univariate risk factors for BHR by odds ratios (OR) with 95% confidence intervals (CI) in two different cut-off points for histamine, $PD_{15}FEV_1 \leq 1.6$ mg (BHR) and $PD_{15}FEV_1 \leq 0.4$ mg ($BHR_{ms}$).

# $FEV_1$ and FVC values were obtained from 291 subjects. Predicted values according to Viljanen *et al.*(1982).
* SPT done for subjects < 61 years of age, n=251.

## 5.3. DETERMINANTS AND RISK FACTORS FOR BHR

Age, gender, family history of asthma and all the determinants that were significant in the univariate analysis were included in the multiple regression analysis. Risks expressed as odds ratios for $PD_{15}FEV_1 \leq 1.6$ mg and $PD_{15}FEV_1 \leq 0.4$ mg based on univariate analyses are presented in Table 10. Results of the multivariate analyses are presented in Table 11.

### 5.3.1. BASELINE LUNG FUNCTION VALUES OF PREDICTED

Baseline $FEV_1$ < 80% of predicted, together with the obstruction ($FEV_1$/ FVC <88% of predicted), yielded an increased risk for BHR (OR 7.16). In case the obstruction was defined as $FEV_1$/ FVC <0.7, the OR was 5.73. Baseline $FEV_1$ < 80% of predicted alone associated with BHR (OR 5.39) and $BHR_{ms}$ (OR 14.26).

In the univariate analysis of lung function variables, $MEF_{50}$ below lower limit of normal (LLN) appeared to be the strongest determinant for BHR and $BHR_{ms}$, and this was especially true when combined that with $FEV_1$ < 80% of predicted. When $MEF_{50}$ < LLN was the only single sign of decreased ventilatory function among all those studied, it was significantly associated with BHR, OR 2.65 (95% CI 1.21-5.82). Among smokers, the risk for BHR was more dependent on $FEV_1$ and on decreased $FEV_1$/ FVC, than $MEF_{50}$ value, as referred to the Study IV, Table 3.

### 5.3.2. EXPOSURE TO TOBACCO SMOKE

#### *5.3.2.1. Smoking*

Of the subjects, 54.8% (n=160) had smoked at least for a year, from whom BHR was determined in 24.4% and $BHR_{ms}$ in 9.4% (Figure 4). Of the current smokers 76.6% had tried to quit smoking.

Smoking increased the risk for BHR as presented in Figure 5. Half a package of cigarettes (5-14) daily yielded an OR by 3.11 (95%CI 1.51-6.42) for BHR. Smoking habits were associated with BHR severity: smoking more than 25 cigarettes daily was a clear risk factor for $BHR_{ms}$ (OR 5.05, 95%CI 1.39-18.35). Starting to smoke at age 13-18 years increased the risk for $BHR_{ms}$ by 4.02 (95%CI 1.08-15.02).

In the multivariate analysis, smoking remained an independent risk factor for BHR and $BHR_{ms}$ even after adjustment of impaired lung function and other determinants that were significant in the univariate analysis (Table 11) (Table 12).

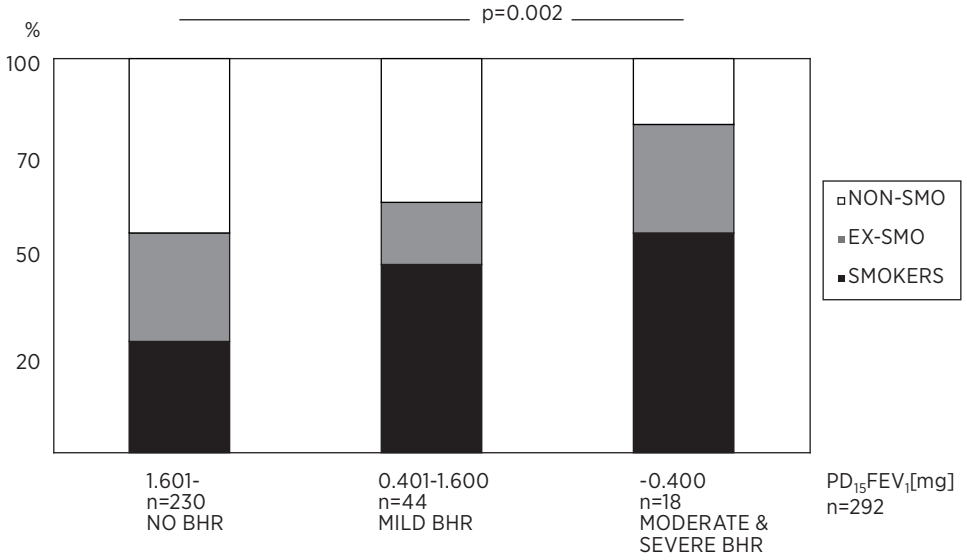



**Figure 4**. Proportion of smokers, ex-smokers and non-smokers in three categories of BHR (marked or severe, mild and no BHR) in the general adult population of Helsinki (n=292).
P-value refers to the trend of smoking habits by the BHR severity classification.

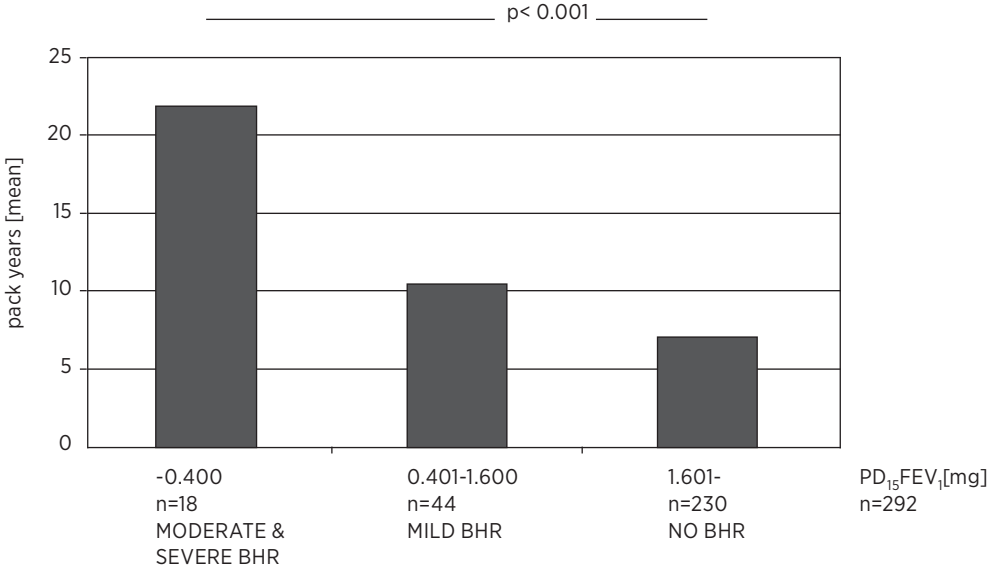



**Figure 5.** Association of pack years of smoking and the severity of BHR.
P-value refers to the trend of increasing number of pack years by the BHR severity.

**Table 11.** Risk in odds ratios (OR) with 95% confidence intervals (CI) for BHR( $PD_{15}FEV_1 \leq 1.6$ mg) and BHRms ( $PD_{15}FEV_1 \leq 0.4$ mg ); the multivariate analyse, all subjects (n=292) .

| | **PD15FEV1 ≤ 1.6 mg** | ETS at work included | **PD15FEV1 ≤ 0.4 mg** | ETS at work included |
|---|---|---|---|---|
| | **OR (95%CI)** | | **OR (95%CI)** | |
| **Age** > 47 years | 0.70 (0.36-1.38) | 0.69 (0.35.1.35) | 0.63 (0.17-2.36) | 0.57 (0.15-2.16) |
| **Men** | 1 | 1 | 1 | 1 |
| **Women** | **2.14 (1.08-4.24)** | **2.09 (1.05-4.16)** | 1.05 (0.31-3.53) | 1.09 (0.32-3.68) |
| **Lung function** FEV1< 80 % of pred | **4.58 (2.07-10.12)** | **4.67 (2.11-10.34)** | **10.75 (3.20-36.11)** | **11.26 (3.28-38.70)** |
| **Family history of asthma** | 1.64 (0.75-3.62) | 1.63 (0.74-3.58) | 1.42 (0.34-5.97) | 1.55 (0.36-6.60) |
| **Allergy (atopy or symptoms of ARC)** | 0.63 (0.33-1.21) | 0.64 (0.34-1.22) | 0.48 (0.15-1.60) | 0.48 (0.15-1.60) |
| **Wheezing or asthma in childhood** | **3.66 (1.22-11.05)** | **3.63 (1.20-10.95)** | 2.18 (0.23-21.11) | 2.17 (0.22-21.09) |
| **Smoking in pack years** | | | | |
| non smokers | 1 | 1 | 1 | 1 |
| <15 years | 0.92 (0.41-2.07) | 0.89 (0.39-2.00) | 1.51 (0.22-10.23) | 1.45 (0.21-9.89) |
| >15 years | **3.87 (1.77-8.43)** | **3.60 (1.62-8.01)** | **9.91 (1.83-53.53)** | **8.28 (1.49-46.00)** |
| **ETS at work** | | **2.02 (1.00-4.10)** | | 2.11 (0.48-9.36) |

**Table 12.** Multivariate analyse of the association of BHR with smoking and lung function data.

| | **PD15FEV1 ≤ 1.6 mg** OR (95%CI) | **PD15FEV1 ≤ 0.4 mg** OR (95%CI) |
|---|---|---|
| Model 1. | | |
| **Lung function** FEV1< 80 % of pred | **5.03 (2.36-10.75)** | **11.35 (3.75-34.36)** |
| **Women** | 1.88 (0.98-3.59) | 0.90 (0.30-2.68) |
| **Wheezing or asthma in childhood** | **3.64 (1.24-10.73)** | 1.65 (0.18-14.82) |
| **Smoking in pack years** | | |
| non smokers | 1 | 1 |
| < 8.5 pack years | 0.68 (0.27-1.72) | 0.48 (0.05-4.98) |
| ≥ 8.5 pack years | **2.63 (1.33-5.18)** | **5.03 (1.31-19.31)** |
| Model 2. | | |
| **Obstruction** FEV1/ FVC < 0.7 | **3.99 (1.59-10.01)** | **12.36 (3.91-39.13)** |
| **Women** | **2.20 (1.16-4.16)** | 1.35 (0.46-4.01) |
| **Wheezing or asthma in childhood** | **3.17 (1.08-9.31)** | 1.32 (0.15-11.54) |
| **Smoking in pack years** | | |
| non smokers | 1 | 1 |
| < 8.5 pack years | 0.75 (0.30-1.85) | 0.57 (0.06-5.88) |
| ≥ 8.5 pack years | **2.68 (1.38-5.23)** | **5.25 (1.36-20.24)** |

#### *5.3.2.2. Exposure to environmental tobacco smoke*

Of the interviewed subjects 41.1% replied that they had never been exposed to ETS at home, 49.7% had been exposed earlier, and 9.2% were currently exposed to ETS. At work, 46.2% had never been exposed to ETS, 45.5% had earlier been exposed, and 8.2% currently. Either exposed at home or at work or both comprised in 73.6%. Women had more frequently been exposed to ETS at home than men (p=0.012).

ETS at home or at work was a risk factor for $BHR_{ms}$; OR 3.73 (95%CI 1.05-13.17) and 4.65 (95%CI 1.32-16.42), respectively. An additive risk was found for ETS, both at home and at work, which yielded a 6.09 fold risk for $BHR_{ms}$ (95%CI 1.95-19.01). The risk was even higher among smokers (OR 7.31, 95%CI 2.72-19.65). Non-smokers were not at risk for BHR, however, regardless of the ETS at home or at work, and a negative smoking history combined with a negative ETS at home and at work did not significantly decrease the risk for BHR (OR 0.75, 95% CI 0.33-1.70).

### 5.3.3. ALLERGIC CONSTITUTION

#### *5.3.3.1. Atopy and multisensitization*

The prevalence of at least one positive SPT was 49% in the studied cohort from the general adult population in Helsinki, and 47% of subjects with BHR were sensitized. The prevalence of positive SPT reactions is presented in Table 13.

Allergic sensitization did not significantly correlate with BHR, nor did any positive reaction to tested single allergens alone (Table 14). Multisensitization (SPT response to ≥ 6 allergens) was significantly associated with $BHR_{ms}$ (p= 0.018), with elevated FENO (> 25 ppb) (p=0.045) and symptoms of allergic rhinoconjunctivitis (ARC) (p=0.003) (Figure 6). The reported symptoms of food allergy yielded an OR of 6.67 risk for BHR (95%CI 2.10-21.19), which was not associated with $BHR_{ms}$.

#### *5.3.3.2. Atopy and airway obstruction*

Atopy combined with obstruction ($FEV_1$/ FVC< 88% of predicted) yielded an increased risk for BHR (OR 6.32). In the multivariate analysis, multisensitization and obstruction remained as independent risk factors for $BHR_{ms}$, OR 4.68 (95%CI 1.10-21.65) and OR 8.29 (95%CI 2.47-27.81), respectively.

**Table 13.** Prevalence (%) of positive SPT reactions.

| | Men (n=110) | Women (n=141) | Total (n=251) |
|---|---|---|---|
| | % | % | % |
| Dog | 22.7 | 17.7 | 19.9 |
| Cat | 22.7 | 15.6 | 18.7 |
| Birch | 16.4 | 21.3 | 19.1 |
| Timothy | 23.6 | 14.2 | 18.3 |
| Mugworth | 11.8 | 12.1 | 12.0 |
| HDM1 | 6.4 | 2.1 | 4.0 |
| HDM2 | 8.2 | 4.3 | 6.0 |
| Any pollen | 35.5 | 29.8 | 32.3 |
| Any animal | 31.8 | 23.4 | 27.1 |
| Any allergen | 50.0 | 44.7 | 47.0 |

HDM1. House dust mite1, Dermatophagoides pteronussinus
HDM2. House dust mite2, Dermatophagoides farinae
No significant difference in the prevalence by gender.

**Table 14.** Allergic sensitization, allergic symptoms and BHR, univariate analysis for histamine $PD_{15}FEV_1 \leq$ 0.4 mg ($BHR_{ms}$) and ≤ 1.6 mg (BHR).

| | PD15FEV1 ≤ 0.4 mg | PD15FEV1 ≤ 1.6 mg |
|---|---|---|
| | OR 95% CI | OR 95% CI |
| **Atopy** | | |
| SPT any positive | 0.8 (0.3-2.1) | 1.0 (0.5-1.8) |
| SPT ≥ 4 positive | 1.7 (0.4-6.2) | 1.2 (0.5-2.9) |
| SPT ≥ 6 positive | **4.7 (1.2-18.8)** | 1.4 (0.4-4.7) |
| SPT cat positive | 2.7 (0.8-9.1) | 1.1 (0.4-2.7) |
| SPT birch positive | 0.6 (0.1-2.9) | 1.0 (0.5-2.2) |

### 5.3.4. TIMING OF THE BHR TESTS

The histamine challenge test was carried out during the birch pollen season (April-June) for 28% (n=83) of those studied. These subjects did not differ in terms of the prevalence of symptoms of ARC. However, those subjects tested in April to June presented an increased risk for $BHR_{ms}$ (OR 2.70, 95% CI 1.03-7.07).

### 5.3.5. FRACTIONAL EXHALED NITRIC OXIDE (FENO)

Mean FENO in the studied population (n=277) was 18.09 ppb (SD 13.47; 2.13-95.60). The association between FENO and BHR was strongly dependent on smoking habits. Only in non-smokers with BHR, FENO was >25 ppb, and significantly higher

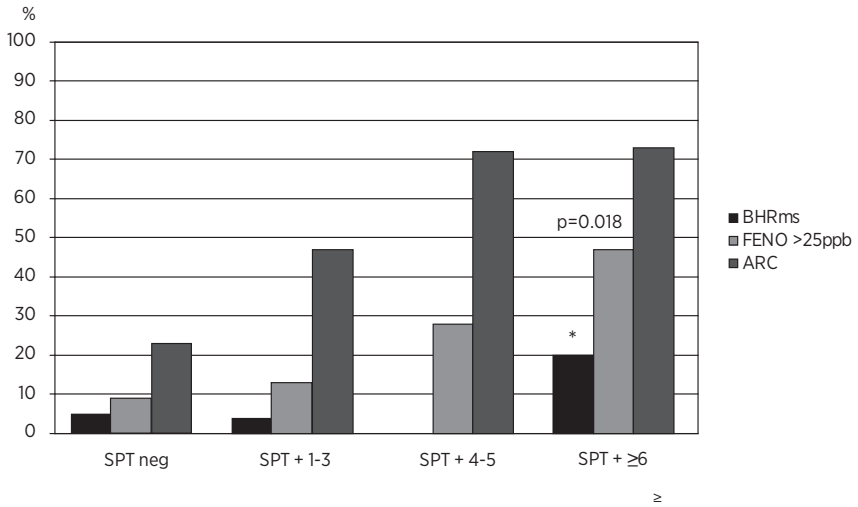



**Figure 6.** Prevalence of BHRms, elevated fractional exhaled nitric oxide (FENO >25 ppb) and reported symptoms of allergic rhinoconjunctivitis (ARC) in different groups of allergic sensitization in terms of number of positive reactions in skin prick tests (SPT).

compared to the remaining subjects (p=0.008). This was not true for smokers or ex-smokers with BHR (Study IV, Figure 2).

FENO was lower among smokers exposed at home and at work compared to the remaining subjects, 13.35 ppb *versus* 19.06 ppb (p=0.008), in contrast, non smokers with no ETS exposure at home nor at work had a higher FENO (22.60 ppb *versus* 17.30 ppb, p= 0.020). Current exposure to ETS was associated with a lower FENO (13.2 ppb) compared to non-exposed (19.3 ppb) (p=0.002).

Subjects who had FENO ≥30 ppb were at more than five-fold risk for BHR when exposed to ETS at work (OR 5.59; 95%CI 1.86-16.81). FENO cut off levels ≥ 25 ppb and FENO ≥ 45 ppb yielded increased risk for BHR, OR 2.98 and OR 5.00, respectively.

### 5.3.6. RESPIRATORY SYMPTOMS

Many of the reported respiratory symptoms associated significantly with BHR (Table 10.) In the group of subjects with $BHR_{ms}$, more significant associations, i.e. higher ORs for symptoms, were found (Table 10).

Seventeen individuals (5.8%) reported asthma or wheezing in childhood. All of them had baseline $FEV_1$ ≥ 80% of predicted, but BHR was found in seven (41%). In those individuals with a wheezing history, father's smoking (65%) was more common than mother's smoking (12 %), (p=0.001). Father's smoking among

**Table 15.** Demographic data of all subjects stratified according to BMI.

| | **All subjects** | | | |
|---|---|---|---|---|
| **BMI (kg/ m2)** | 26 (17-55) | <25 | 25-30 | >30 |
| No. of subjects | **292** | 139 | 105 | 48 |
| % of subject | **100** | 48 | 36 | 16 |
| Age mean (range) | **46 (26-66)** | 44 (27-65) | 48 (27-65) | 48 (26-66) |
| Men (%)// Women (%) | **42// 58** | 39// 61 | 48// 52 | 40// 60 |
| Fam Hist Asthma (%) | **18** | 20 | 16 | 17 |
| Physician dg.asthma (%) | **5** | 4 | 5 | 6 |
| **LUNG FUNCTION** | | | | |
| $FEV_1$ [L] mean (range) | 3.37 (1.71-5.90) | 3.40 (1.84-5.90) | 3.45 (1.88-5.47) | 3.10 (1.71-4.55) |
| FVC [L] mean (range) | 4.34 (2.15-8.03) | 4.37 (2.68-8.03) | 4.47 (2.39-7.46) | 3.98 (2.15-6.27) |
| $FEV_1$ < 80% of pred (%) | **13** | 14 | 9 | 19 |
| $FEV_1$/ FVC < 0.7 (%) | **7** | 9 | 6 | 2 |
| **SMOKING** | | | | |
| Non smokers (%) | **41** | 48 | 33 | 42 |
| Ex smokers (%) | **26** | 18 | 33 | 31 |
| Current smokers (%) | **33** | 35 | 33 | 27 |
| **BHR_Histamine $PD_{15}FEV_1$** | | | | |
| ≤ 0.400 mg (%) | **6** | 6 | 7 | 6 |
| 0.401-1.600 mg (%) | **15** | 14 | 14 | 19 |
| >1.601 mg (%) | **79** | 80 | 79 | 75 |

Predicted values according to Viljanen *et al.* (1982).

the childhood wheezers compared to the remaining of the studied did not differ (p=0.379). Mother's smoking did not associate with BHR.

Longstanding cough did not associate with BHR, but cough in the mornings yielded a 2.17 fold risk for BHR, and ever wheezing increased the risk to 2.88 fold (95%CI 1.60-5.18). Nocturnal asthma symptoms, SOB/wheezing during the past 12 months, and reported SOB/wheezing induced by air pollution, increased the risk for $BHR_{ms}$ by 6.83 (95%CI 1.93-24.19), 5.74 (95%CI 1.66-19.88), and 5.16 (95%CI 1.66-16.01), respectively.

### 5.3.7. PHYSICIAN DIAGNOSED ASTHMA AND USE OF ASTHMA MEDICATION

The prevalence of physician diagnosed asthma was 4.5% (n=13), of whom 69.2% were smokers or ex smokers. $FEV_1$/ FVC< 0.7 was found in 8.6% (n=25) of the subjects of whom 2 had an asthma diagnosis. $FEV_1$/ FVC<0.7 after

bronchodilattaion test was found in 3.8% (n=11). Eight subjects (2.1%) reported their physician diagnosed chronic bronchitis, whereas reported symptoms for chronic bronchitis indicated of a prevalence of 7.9% (n=23). Physician diagnosed asthma was associated with $BHR_{ms}$ (OR 5.28, 95%CI 1.31-21.22), whereas chronic bronchitis, neither physician diagnosed nor based on reported symptoms, did not significantly associate with BHR.

Of those subjects with recent use of asthma medication, 27% reported physician diagnosed asthma, and a half of them did not show BHR. No subjects with earlier asthma medication had severe BHR. Recent use, and ever use of asthma medication was associated significantly to BHR, OR 4.29 (1.76-10.45) and OR 2.11 (1.08-4.09), respectively.

#### 5.3.8. OBESITY

Sixteen percent of the subjects were obese (BMI > 30 $kg/m^2$), among whom the $FEV_1/FVC$ ratio was ≥0.70 except in one. Table 15 presents the demographic data of subjects stratified according to BMI. BMI and BHR did not associate significantly.

## 5.4. INCIDENT ASTHMA IN THE STUDY POPULATION 1996-2007 (STUDY II)

### 5.4.1. INCIDENT ASTHMA IN 11 YEARS' FOLLOW-UP DEFINED BY POSTAL SURVEYS

#### *5.4.1.1. Changes in the prevalences 1996-2007 (Study II)*

The prevalence of physician diagnosed asthma, use of asthma medication, and reported symptoms of ARC had increased by the 11 years' follow up, which was not true for respiratory symptoms. Regardless of aging in this cohort, shortness of breath (13% *versus* 15%), for example, did not significantly increase.

In 2007, ARC was reported by 41% of subjects, and both physician-diagnosed asthma and use of asthma medication by 9%. The greatest increase in prevalence was observed in symptoms of ARC, from 38% to 41%, whereas a 3% increase existed in the asthma diagnosis and use of medication. Current smoking decreased significantly over the follow up time, from 31% to 23%. The greatest decrement of 13% was seen in the group of young smoking women (31-40 years). The women aged 41-80 years presented only a 7% decrement, and the decrement in men was even less, 5% on average.

#### 5.4.1.2. Incidence of asthma

In the general population cohort of the FinEsS-Helsinki study (4302 individuals), a 4.0% cumulative incidence was found during the 11 years' follow up from 1996 to 2007. These 157 individuals with new adult onset asthma corresponded to an incident rate of 3.7/ 1000/ year among those 3966 subjects who constituted the cohort at risk for asthma in 1996.

When those subjects with asthma medication, physician diagnosed chronic bronchitis, or COPD were excluded from the population at risk in 1996 (n=3821), 132 new adults onset asthmatics remained, leading to an adjusted incident rate of 3.2/ 1000/ year, among men 2.5/ 1000/ year and in women 3.7/ 1000/ year, p=0.026. If subjects with recurrent wheeze or shortness of breath were also excluded from the cohort at risk, the incidence rate was 2.5 / 1000/ year, 1.7 for men and 3.1 for women (p=0.008), respectively.

In 2007, remission of asthma occurred in 43 (17%) of those 254 individuals, who reported active asthma in 1996. Of these 43 individuals, 11 men and 32 women were free from symptoms and did not use asthma medication.

#### 5.4.1.3. Incidence of allergic rhinoconjunctivitis (ARC)

New adult onset cases of ARC occurred in 447 individuals, a majority of whom were women (274 *versus* 173), p=0.012. The annual crude incident rate was 16.8/ 1000 persons. Incident ARC was greatest in the age group 31-40 years. Of these youngest individuals, 23% reported development of ARC over the 11 years's follow up, whereas only 14% of those were in the oldest age group of 71-80 years. Living in the countryside or on a farm in early childhood (<5 years) was associated with a lower incidence of ARC (p=0.022 and p=0.008). Smoking was not significantly associated with incident ARC. Family history of ARC yielded an increased risk for incidence of ARC by 1.73 (95%CI 1.34-2.25). In a multivariate model. ARC was an independent risk factor for incident asthma.

In 2007, remission of ARC was reported by 18% (n=296) of those 1635 individuals who reported ARC in 1996. More men were in remission compared to women (21% *versus* 17%, p=0.06).

#### 5.4.1.4. Determinants of incident asthma

Aging was associated with an increased risk for incident asthma above the age of 61 years, leading to a more than two-fold risk in senior citizens (OR 2.33; 95%CI 1.30-4.18). A similar level for an increased risk was found in association with female

gender, family history of asthma, history of allergic rhinoconjuctivitis, and long-term smoking (Study II, Table 4). When individuals with asthma medication and COPD were excluded from the population at risk, i.e. presenting the adjusted risk, the determinants for incident asthma remained. With this adjusted criteria, a status of being an ex-smoker yielded the highest risk for incident asthma (OR 2.5, 95%CI 1.35-3.16).

# 6 DISCUSSION

## 6.1. DISCUSSION OF THE MAIN RESULTS OF STUDIES I-IV

### 6.1.1. METHODOLOGY OF MEASURING BHR

In the present studies, BHR was assessed with inhaled histamine diphosphate or methacholine by using a nebulization method with controlled tidal breathing (Nieminen 1992, Sovijärvi *et al.* 1993). The methods, which have not been compared before, showed good repeatability. Both the histamine and methacholine methods have been used, however, in paediatric and adult respiratory research (Helenius *et al.* 2002, Sovijärvi *et al.* 2003, Kyllönen *et al.* 2006, Malmström *et al.* 2008, Lindström *et al.* 2009, Malmberg *et al.* 2010) and commonly in clinical diagnostics in Finland.

The present histamine test (Sovijärvi *et al.* 1993) has been evaluated in comparison with other direct airway challenge methods by Chinn and Schouten (2005). Its high intraclass correlation coefficient (0.95) was the best in the comparison with other 23 BHR methods reported in this review. The inhalation synchronized tidal breathing and the optimal composition of the histamine and methacholine particles (mass median diameter 1.6μm, size 75% of the particles 1-3 μm) leads to less non-uniform distribution of the constrictor to central and peripherial parts of the airways than would larger particles.

Deposition studies of the metacholine test (Nieminen *et al.* 1987) demonstrated that alveolar deposition in asthmatic subjects was 43% of the output of the nebuliser. The deposition of radiolabelled methacholine exhibited a 17% increase in the lung deposition in healthy subjects when the nebuliser was operated in an early phase of tidal volume, 80ml from the beginning of inspiration, used in the present studies *versus* 320 ml from the beginning of inspiration. Inspiratory flow rate was set to be slow, 0.5 L/s and tidal volume 0.5 L in order to improve the reproducibility of the consecutive measurements (Nieminen *et al.* 1987), i.e. to avoid changes in bronchomotor tone due to the inspiratory manouvere (Lim & Ang 1997, Sinard *et al.* 2005, Cockcroft 2008, Prieto *et al.* 2008).

In Study I (Kemi, North Finland), the choice of subjects selected for the comparison was of clinical interest, which met the aims of the Brussel declaration in 2004 for high-priority studies in terms of asthma and respiratory diseases (Holgate

*et al.* 2008). The subjects included presented a random sample of individuals, the majority of whom were symptomatic, but did not have asthma or chronic bronchitis.

The results of Study I affirmed that the methods were in good agreement in staging the severity of BHR (weighted kappa 0.64; CI 95% 0.46-0.82). Baseline $FEV_1$ level did not significantly affect the agreement.

Values of the baseline $FEV_1$ measurements, before both the methacholine and histaminen tests, were evaluated prior to the final selection of the cases for the further comparative analysis. The results in $PD_{15/20}FEV_1$ are dependent on the percentage change from the baseline $FEV_1$. In order to study the agreement of these two methods, the baseline $FEV_1$ values did not differ more than 10% between the metachcoline and histamine testing days. This obviously resulted in a selection, in which those subjects with the greatest intra-individual $FEV_1$ variability were excluded from the final study cohort. It is possible that this standardization manner excluded some of those subjects who potentially would have presented significant BHR. However, it did not affect the agreement between the studied BHR methods.

The histamine and methacholine test were carried out seven days apart. The order of testing did not have a significant association to the results. Furthermore, the majority of subjects were tested during the morning hours (mean at 11 am), however the timing of the BHR measurements did not influence the results of agreement. Tachyphylaxis of methacholine has been reported to last over for 4-7 days (Fujimura *et al.* 1999, Sinard *et al.* 2005), however histamine has not been shown to accumulate or decrease in response in serial measurements (Sovijärvi *et al.* 1993, Sinard *et al.* 2005).

In addition to the original cut off levels for histamine $PD_{15}FEV_1$ and metacholine $PD_{20}FEV_1$ (Nieminen 1992, Sovijärvi *et al.* 1993), different $PDFEV_1$ cut-off levels were investigated. The agreement of methacholine and histamine test results was inversely related to the PD values; better agreement was found in more pronounced BHR, i.e. in subjects with clearly sensitive airways to direct stimuli. Since subjects with diagnosed asthma were excluded, the majority of patients did not show abnormally increased BHR when using the clinically validated points of abnormality. We found better agreement between $PD_{15}$ histamine (< 1.6 mg) and $PD_{15}$ methacholine (< 2.6 mg) than with $PD_{15}$ histamine (< 1.6 mg) and $PD_{20}$ methacholine (< 2.6 mg). This is in accordance with the results by Malo *et al.* (1983), they suggested a lower $FEV_1$ cut of level (-6%) to be used in general population studies of BHR. Our results on the lnDRS did not increase the value of our findings gained by the $PD_{15}FEV_1$ method.

The histamine test induced slightly more respiratory symptoms than the metacholine test. This is line with earlier studies (Juniper *et al.* 1978, James & Ryan 1997). Extrathoracic airway obstruction or irritable laryngeal reactivity might constitute observed respiratory symptoms (Bucca *et al.* 1995a&b, Rolla *et al.* 1997). In Study I, expiratory wheezing, as a sign for peripheral obstruction, accounted in a similar degree during both provocation tests. In accordance with this, there is

evidence from a recent study from Leuven, Netherlands (Janssens *et al.* 2012) that symptom perception during a histamine challenge test could predict upcoming problems with asthma treatment.

In Studies I, III, and IV, the number of subjects was relatively small. The representativeness of the present cohort for the BHR-Helsinki study (Studies III and IV) was compared to the original study cohort of FinEsS I postal survey by gender and age, and the replies of the postal survey (Pallasaho *et al.* 1999). In the present study cohort the physician diagnosed chronic bronchitis (question 4) and symptoms related to chronic bronchitis (questions 8A and B) were slightly less prevalent than in the original study population, but prevalences of reported respiratory symptoms, symptoms of allergic rhinoconjunctivitis (ARC), physician diagnosed asthma, and smoking were alike.

### 6.1.2. PREVALENCE OF BHR

In the general adult population of Helsinki, the prevalence of BHR was 21%, and severe or moderate BHR was found in 6% of the studied subjects, respectively. In Kemi the prevalence of BHR and BHRms were of a similar level, although assessed in a randomly selected adult cohort of general population without physician diagnosed asthma or chronic bronchitis. Both these results agree with the range of BHR found in the European Community Respiratory Health Survey (ECRHS), where the prevalence of BHR varied from 3-28% in 16 countries, with a median prevalence of 13 % (Chinn *et al.* 1997). Referred by Jansen (1999) the prevalence of 11 BHR population studies published in 1984-1994 presented the variation from 6-35%. All these percentages of prevalence are in accordance, but show large variation. These findings indicate no increase in BHR in the general population during the past three decades, which is in line with an observed decline in BHR's prevalence found in the 11 year's follow up in the SAPALDIA study (Curjuric *et al.* 2011). The diversity of these presented percentages may be explained by the different methods and study designs employed.

Cohorts may differ significantly from each other. Furthermore, the inclusion criteria for the subjects vary, which causes variation in the reported prevalence and outcomes. In the Swiss SAPALDIA study, the prevalence of BHR was 13% (Curjuric *et al.* 2011), however the inclusion criteria for the BHR test was different from ours, restricting more subjects out of the study cohort by excluding subjects who reported use of asthma medication, had a baseline $FEV_1$ <70% or $FEV_1$/ FVC <80% of predicted, or had a post saline drop >10% of the maximum baseline $FEV_1$ value. The last mentioned was not even measured by our BHR protocols. The Normative Aging study (Sparrow *et al.* 1987 &1991) included only healthy men, thus not representing a general population. Regardless of the BHR method in use and the proportion

of hyperreactive subjects, the percentages of symptomatic individuals show great variation, as listed in Table 3.

The prevalence of asymptomatic subjects with BHR was lower here than reported in the previously mentioned SAPALDIA study (12% *versus* 51%), which indicates the usefulness and better sensitivity of the challenge method used here in general population studies (Sovijärvi *et al.* 1993). Reporting the prevalence of asymtomatic BHR faces obstacles due to the different criteria presented for an asymptomatic subject. As previously described (Rijcken *et al.* 1987), asymptomatic subjects were defined here based on a questionnaire (Kainu 2008, p.47-48).

Histamine and methacholine provocations have been compared from different viewpoints with other provocation methods (Anderson *et al.* 2002, Sterk 2002, Joos *et al.* 2003, Choi *et al.* 2003, Anderson & Brannan 2003, de Meer *et al.* 2004). Some studies have described indirect methods such as hypertonic saline or mannitol to be more specific for asthma (Andersson *et al.* 2002 & 2003), typically in comparison to measures of acute eosinophilic inflammation found in asthmatic patients. The directly acting bronchoconstrictor agents, however, have been regarded as more sensitive in diagnosing asthma than the indirect stimuli (Joos *et al.* 2003, Koskela *et al.* 2003a&b). In terms of physician diagnosed asthma, the cut off level for $BHR_{ms}$ used here gave the same prevalence for physician diagnosed asthma as reported by the postal survey preceding (Pallasaho *et al.* 1999). The presented histamine test is thus suitable in assessment of BHR in population studies.

### 6.1.3. DETERMINANTS OF BHR

#### *6.1.3.1. Decreased $FEV_1$*

The decreased $FEV_1$ was the main determinant for an increased BHR in the present studies. Spirometric values were employed for prediction in the regression analysis, where the height and weight of the participants were adjusted.

These results indicate that airway obstruction at baseline, defined as $FEV_1$/ FVC < 88% of predicted or $FEV_1$/ FVC < 0.7, causes a 4-fold higher risk for BHR, and increases to 7-fold when combined with a decreased $FEV_1$ value. The risk of decreased ventilatory functions was doubled among the smokers. As a sign for early airway closure, the $MEF_{50}$ below LLN independently associated with an increased risk for BHR and $BHR_{ms}$, at the same magnitude as a decreased $FEV_1$ value. Results from others, also assessed in a general adult population cohort, report of this close relation of decreased $FEV_1$ and increased BHR (Rijcken *et al.* 1988, Jansen *et al.* 1997, de Marco *et al.* 1998, Schwartz *et al.* 2002, Scichilone *et al.* 2005), but no

such results by the $MEF_{50}$ parameter and BHR to histamine in an adult general population have been published.

#### 6.1.3.2. Gender

In Study III, gender and the BHR were not associated, similar to the findings reported by PausJenssen and Cockcroft in 2003, but contradictory to some other studies (Malo *et al.* 1983, Jögi *et al.* 2004).

Gender difference has been observed in studies on prevalence and incidence of asthma (Leynaert *et al.* 1997, Chinn *et al.* 2006, Leynaert *et al.* 2012). The determinants for this remain unanswered, as for BHR. Curjuric *et al.* (2011) demonstrated that the increase in BHR slope appeared in women 10 years earlier in age in comparison to men, but it was not associated with increase in the weight in women. Adipose tissues in women (Leynaert *et al.* 2012), improved diagnostic methods, and increase awareness of asthma (Lundbäck *et al.* 2001) have been under discussion as potential contributing factors. The use of fairly old reference values for ventilatory parameters (Viljanen *et al.* 1982, Quanjer *et al.* 1983) could impact on the number of false positive cases of asthma, which has not been discussed in detail in the literature. Thus, overall, the gender effect remains to be elucidated.

#### 6.1.3.3. Respiratory symptoms and BHR

BHR was associated with many respiratory symptoms and irritants in this study, which is in accordance with the known nature of BHR in children (Salome *et al.* 1987, Pattemore *et al.* 1990, Malmberg *et al.* 2008) and in adults (Salome *et al.* 1997, Rijcken *et al.* 1987, Sparrow *et al.* 1987, Woolcock *et al.* 1987, Burney *et al.* 1994, Xu *et al.* 1997, Jansen *et al.* 1999a). The symptoms alone cannot reliably explain BHR, as presented in Table 3. Most of the respiratory symptoms were related to mild BHR, and much fewer symptoms seemed to be related to $BHR_{ms}$, which probably reflects to the better asthma positive predictive value, especially when it concurs with a low cut off value for BHR (Koskela *et al.* 2003a&b).

In the present study, long-lasting cough was not associated with BHR in adults, as found by de Marco *et al.* (1998). Cough seems not to be a typical symptom of BHR. This differs from the pediatric patients, in whom cough is more frequently associated with BHR, and it is the most sensitive finding for wheezing and respiratory tract illness in children (Rivera-Spoljaric *et al.* 2009).

#### *6.1.3.4. Smoking*

A dose-dependent pattern of smoking habits was demonstrated in this assessment of BHR in the general adult population. This data evidently corresponds to the fact that increasing smoking habits associate with marked BHR. ETS at home, and especially at work, are risk factors for BHR, in which the smokers had the highest risk for BHR. These findings regarding smoking and ETS are in agreement with the data of earlier studies by Chinn *et al.* (2005), Schwartz *et al.* (2002) and Janson *et al.* (2001).

A third of continuous smokers had started in their teenage years, and a third of them had BHR, whilst a tenth presented BHRms. Starting to smoke at early age of 7-14 years, increased the risk for $BHR_{ms}$ significantly (OR5), and starting smoking when a teenager (15-19 years) doubled the risk for BHR in adulthood. Studies of exposure to tobacco smoke indicate that the time and length of the exposure both impact on ventilatory function and BHR (Janson *et al.* 2001 & 2006, Chinn *et al.* 2005, Chaudhuri *et al.* 2006), in addition to the acute inflammatory reactions triggered on the respiratory epithelium (Barnes 2008 & 2009, Louhelainen *et al.* 2010).

Smoking leads to remodelling changes on the airways, similar to those changes observed with asthma (Karjalainen *et al.* 2000, Vignola *et al.* 2000, Colice 2002, Chetta *et al. 2003*). According to the recent findings by Tsurikisawa *et al.* (2010), the presented histamine test could serve well as a measure for changes of airway remodelling. Furthermore, they established that BHR to histamine, but not Ach, was associated with histopathological changes typical for airway remodelling. They examined 50 adult patients with severe asthma, but no smokers. They found that ASM thickness was inversely correlated with BHR to histamine but not to Ach, thus indicating that their final conclusions could be applied to remodelling changes typical for a long duration of smoking as well. Willemse and colleges (2004) have earlier shown that a one-year smoking cessation improved both direct and indirect airway hyperresponsiveness in COPD, thus supporting the role of BHR measurements also in ever smokers.

#### *6.1.3.5. Severe respiratory infection, wheezing or asthma in childhood*

The results presented here have revealed that respiratory problems in early childhood, which cause wheezing, or a post infectious sensitivityof the airways after a severe respiratory infection, are associated with BHR in adulthood.

Other epidemiological studies have also made these arguments (Sunyer *et al.* 1997, Sears *et al.* 2003, Vonk *et al.* 2004, Toelle *et al.* 2004, Porsbjerg *et al.* 2006), however determinants of causality, a pathway of pathophysiological factors from

early childhood to adulthood and aging, have not been fully elucidated (Prescott & Nowak-Wegrzyn 2011).

Viral infections such as RSV and adeno virus have been associated with increased risk for BHR and asthma in childhood (Lemanske *et al.* 2005, Stein *et al.* 1999, Kotaniemi-Syrjänen *et al.* 2007). The recent immunopathological studies on respiratory diseases indicate that the inflammatory Th1/ Th2 pathways interact following a virus infection (Strickland and Holt 2011). Young atopic individuals exhibit the highest the risk for asthma (Holt *et al.* 2010). Animal models suggest that variation on virus-host interactions exist (Sutton *et al.* 2007), and some viruses are able to cause the final inflammatory response by modulating the actions of high-affinity IgE receptor on lung dendritic cells (Grayson *et al.* 2007). These interactions, and modulation of inflammatory responses, could be studied in older individuals, among whom BHR is believed to be increased (Scicilone *et al.* 2005), but skin reactivity is less frequent (Cline & Burrows 1989).

Results of the present study indicate that wheezing below the age of five results in an increased risk for at least mild BHR in adulthood, which has to be considered in planning and publishing new treatment guidelines for baby wheezers (Panickar *et al.* 2009). There is no available data on longitudinal studies that negate a long-term effectiveness of anti-inflammatory treatment in childhood asthma or recurrent wheeze in terms of current or incident BHR in adulthood (Jartti *et al.* 2007).

#### *6.1.3.6. Rhinitis*

In the cross sectional FinEsS-Helsinki (Study III), no symptoms were evident for allergic rhinoconjunctivitis (ARC) that has the potential to increase the risk for BHR. Allergic rhinoconjuctivitis doubled the risk for incident adult on-set asthma (Study II), as reported by other's (Boulay & Boulet 2003, Shaaban *et al.* 2008).

Rhinitis and sinusitis are known to be associated with BHR, even in the absence of asthma symptoms (Henriksen *et al.* 2001) or physician diagnosed asthma (Rolla et al. 1997). As found by Shaaban (2008), rhinitis and BHR have been defined independent risk factors for incident asthma. The mutual effects of rhinitis and BHR have not fully been established, however. Studies on rhinitis as a risk factor for BHR are scarce. In an 8-year follow-up, Shaaban *et al.* (2007) found 10% cumulative incidence of BHR among subjects with allergic rhinitis, 7% for subjects with atopy without rhinitis, and 5.5% for subjects negative for both these conditions. Of those individuals who had BHR at baseline (7%), 35% of them carried allergic rhinitis. Remission of BHR in follow up was increased among those receiveing nasal-steroid medication (OR 0.33; 95% CI 0.14-0.75).

Subjects BHR tested in April to June had an increased risk for BHR in the present study, regardless of the atopic constitution or symptoms of ARC. This is in

accordance with results by Madonini *et al.* (1987), who reported a seasonal increase in BHR in rhinitis patients. Subjects with rhinitis and BHR have been reported to express distinct in inflammation markers, including EBC, pH, EBC nitrite, and FENO, which have been suggested to serve as biomarkers for identifying rhinitis patients at risk for developing asthma (Skiepko *et al.* 2011).

#### *6.1.3.7. Allergic sensitization*

The decision of the age limit for SPTs only for individuals under 61 years was based on earlier data on decreased skin reactivity in later adult life (Cline & Burrows 1989). The lack of SPT results in individuals over 61 years led to neglation of some these results, thus probably decreased statistical power of some the findings reported here. From this point of view, the age of studied subjects has been a limitation in the ECRHS studies as well. In those studies, the subjects have been 20-44 years at the baseline (1990-1994), and the ECRHS I and II reports do not include data of the sensitization and BHR in subjects over 61 years either (Harrop *et al.* 2007, Gunnbjörnsdóttir *et al.* 2009).

Allergic multisensitization was significantly associated here with BHR response to histamine, while sensitization to one or a few allergens was not. This is in line with the findings by Kerkhof *et al.* 2003, in which only high levels of specific IgE for indoor allergens, i.e. multisensitization, associated with BHR. This is in accordance with a large general population study from the Vlagtwedde and Vlaardingen study in Netherlands where the high serum total IgE levels (< 100 kU/L) associated with BHR both in symptomatic and asymptomatic individuals (Jansen *et al.* 1999a). They also found that skin test positivity associated with BHR only in symptomatic subjects, and these findings agree with results by Kerkhof *et al.* (2000) of the ECRHS study, in which low levels of specific IgE to indoor allergens associated with symptoms only if BHR existed. Results by Patelis and colleges (2012) indicated that the risk for asthma increases with multiple sensitisations to different allergen groups.

In the present cohort, the prevalence of at least one postive SPT reaction was 49%. Earlier studies, such as a study with younger male conscripts (Haahtela *et al.* 1980), and another study with university students (Kilpeläinen *et al.* 2001), indicate great variation from 30% to 70% of a single positive SPT reaction. The younger cohorts present higher percetages, as do symptomatic, in comparison to asymptomatics (Haahtela *et al.* 1980, Kilpeläinen *et al.* 2001, Pallasaho *et al.* 2006). Geographical factors may explain some diversity in the reported percentages observed, as has been reported of the appearace of a single positive SPT in between two otherwise similar cohorts from Karelia, one from the Finnish and the other one from the Russian side of border (Vartiainen *et al.* 2002). In the present cohort, the prevalence of non-atopic subjects who reported ARC symptoms constituted in 32% of all those

subjects who reported ARC, which is regarded as a high percentage. This finding should be interesting to confirm and to study further, also in a longitudinal setting.

Recent publications in allergy raise the interest in explaining airway hyperresponsiveness by immunological hyporesponsivess (von Hertzen *et al.* 2009, Joenväärä *et al.* 2009), which then could link the allergen induced symptoms of rhinoconjuctivitis and multisenzitation to BHR as found here, and as has been reported by others (Spallarossa *et al.* 2003) among polysensitized teenagers. Responses to allergen exposure on nasal respiratory mucosa have been shown to activate the cell caveolar transit system in a different fashion for atopic and non-atopic subjects, in which the pathology and symptoms are associated to hyporeactivation of the intracellular systems involved (Renkonen *et al.* 2009, Mattila *et al.* 2010). This could explain individual differences in the response to spring pollen season, where atopic subjects may also react to different degrees. Boulay & Boulet (2002) has suggested that a well functioning adaptation process in allergic rhinitis subjects may protect them from developing asthma, which might explaining some of these inter-individual differences as well.

#### *6.1.3.8. Fractional exhaled nitric oxide (FENO)*

Exhaled nitric oxide (FENO) leveled the BHR severity only among the non-smokers, but BHR and FENO were not associated in the general adult population in Helsinki. Among all those studied, the FENO level was highly associated with the allergic constitution, defined as number of positive SPT reactions and reported symptoms of ARC. Similar kinds of results have been reported by Ekroos *et al.* (2009) and Rouhos *et al.* (2005), who have studied young male non-smoking conscripts with mild asthma.

These findings support the fact that smoking can reduce FENO by different mechanisms (Silkoff *et al.* 2000, Török & Leuppi 2007). Results by Rouhos *et al.* (2010) suggested that smoking attenuates the increase in NOexp in atopic, but not in non-atopic young adults with asthma. In our study a different age group was studied.

#### *6.1.3.9. Obesity*

In the Studies III-IV, obesity (BMI>30) was not significantly associated with BHR. We found that the proportion of subjects with $FEV_1$ below LLN was the same in the three BMI categories. However, the baseline values for $FEV_1$ [L] and FVC [L] were significantly lower among the obese (BMI > 30) compared to less obese subjects. Of the obese, all except one subjects had the ratio $FEV_1$/ FVC >0.7, and none of the obese had $FEV_1$ and $FEV_1$/ FVC of predicted below LLN.

Much speculation exists about the association of asthma and obesity, whether an increased risk really exists, or is it just confounding factors that result in positive conclusions (Pistelli *et al.* 2008). In the majority of the studies on BHR and obesity, no significant association has been found (Schachter *et al.* 2001, Aaron 2008, van Veen *et al.* 2008), even though an increased risk for asthma has been presented (Schachter *et al.* 2001). It is common to present that the obese and women, often co-presenting as obese women, exhibit a higher prevalance or an increased risk for asthma and BHR (Leynaert *et al.* 2012, Dixon *et al.* 2010, Shore 2010, Chinn *et al.* 2006). As stated by Török and Leuppi (2007) and Kanner *et al.* (1994), the size of airway calibre is an important determinant of BHR. Thus, studies that assess the mechanical factors and lung function measurements in association with BHR, both in obesity and in subjects with low lung function values ($FEV_1$ and FVC), are required.

#### *6.1.3.10. Heridity*

Neither parental asthma, nor sibling asthma, increased the risk of BRH in this study population. Results of the Italian Po Delta epidemiological study published Kurzius-Spencer *et al.* (2004) found a significant father-son correlation of the metacholine dose response curve, but no evidence of a major gene causing the BHR itself.

The genetics studies of BHR have been of minor contribution so far. The linkage studies and positional cloning strategies have so far discovered genes, which are expressed in epithelial cells and smooth muscle cells (Guy Brussell, personal communication, European Respiratory Society Annual Conference, Post Graduate course 5, 2011). BHR has been associated with ADAM33, which encodes disintegrin and metalloproteinase 33 (Van Eerdewegh *et al.* 2002), but the genetic background of BHR remains unclarified. Studies of pooled data bases of blood samples from asthmatics and controls are under investigation.

### 6.1.4. INCIDENCE OF ASTHMA IN FINLAND

Results of the 11years (1996-2007) follow up indicate no further increase the incident asthma in Finland. A 4.0% cumulative incidence was evident in this general population cohort of 4302 individuals during the years 1996-2007. The annual incidence rate of asthma is in line with the results from neighbouring Nordic countries (Ekerljung *et al.* 2008, Torén *et al.* 2004)(Figure 7).

Figure 7 illustrates results drawn together from 4 different incident asthma studies conducted in Sweden and Finland during the years of 1986-2007. Studies included in Figure 7 are OLIN 1986-1996 (Lundbäck *et al.* 2001 ), RHINE 1990-2000 (Torén *et al.* 2004), FinEsS-Stockholm 1996-2006 (Ekerljung *et al.* 2008),

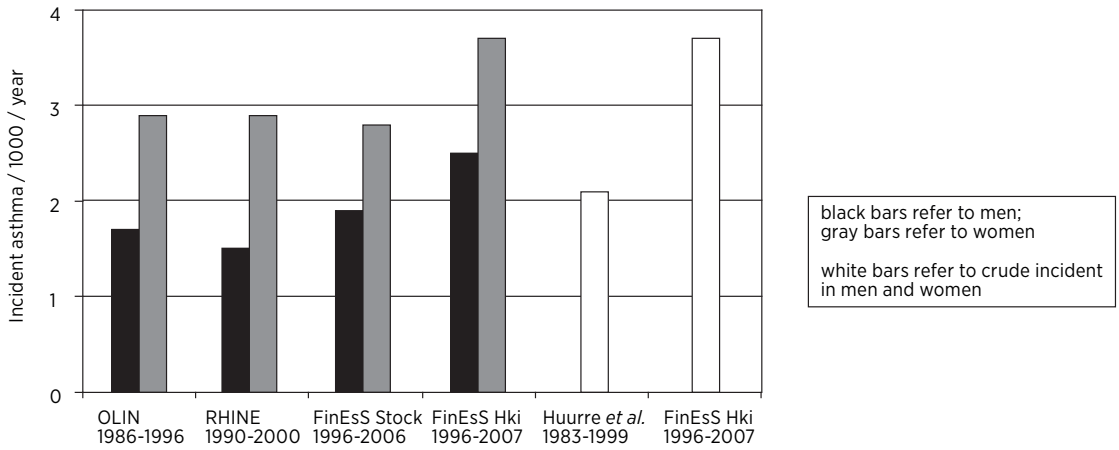



**Figure 7.** Incident rates of asthma. (modified from Ekerljung *et al.* 2008)

FinEsS-Helsinki 1996-2007 (Study II) and Huurre *et al.* 1983-1999 (Huurre *et al.* 2004). Data presented are comparable due to the same criteria and same questions applied to define population at risk and incident asthma cases.

The first 4 double-bars present numbers for adjusted indicent/ 1000/year among men and women, respectively. The studies present a significant gender difference in the rates for incident asthma $p=0.01$, $p<0.01$, $p=0.06$, and $p=0.026$, respectively.

#### *6.1.4.1. Incidence rates*

In Figure 7, the two columns from the right, present incidence rates of asthma in two different Finnish cohort studies. Huurre *et al.* 2004 presents data of 16-32 years old individuals, where as the age range in the FinEsS-Helsinki cohort was 20-69 years at the beginning of the study in 1996. In respect to the presented incidence rates, the two different Finnish asthma studies differ significantly in the rate for incidence of asthma, 3.7/1000/ year (FinEsS Hki, Study II) *versus* 2.1/1000 /year (Huurre *et al.* 2004). This could be explained by the difference in the ages of studied (20-69 years *versus* 16-32 years) and by possibly the different years of conducting these studies (1996-2007 *versus* 1983-1999). Both these facts represent such determinants, which ought to be taken in consideration when evaluating and comparing results from incident asthma studies.

#### *6.1.4.2. Risk factors for incident asthma*

The main risk factors for incident asthma have not changed in Finland over the years. Heritability and atopy play a major role for incident asthma. In addition to the family history of asthma and rhinitis, environmental factors are also strongly contributive, including living surroundings and exposure to tobacco smoke. Female gender was associated with an increased risk for incidence of asthma. Except for the effect of aging, these results are concomitant with the findings of the others' (Ekerljung *et al.* 2008). These results support the actions made worldwide over recent years to reduce incidence of allergic and tobacco related diseases (WHOstatement 2002).

## 6.2. EPIDEMIOLOGICAL CONSIDERATIONS OF BHR MEASUREMENTS

### 6.2.1. STUDY COHORT AND ITS EFFECTS ON FINAL OUTCOMES IN EPIDEMIOLOGICAL BHR STUDIES

#### *6.2.1.1. Sample size*

For an epidemiological study setting, the number of individuals included in the present studies was small, but acceptable. Some results of the logistic regression exhibited a wide 95% CI, but indicated that the result is based on a small number of cases, and thus recognizeable while reading and interpreting the results.

#### *6.2.1.2. Selection*

The role of BHR testing in asthma prevalence surveys has been discussed (Pearce *et al.* 2000). It is partly to due the selection procedure for a BHR study, in which only individuals that fulfill the inclusion criteria for BHR testing are included, and still considered to present a random sample of general population. In addition to the reported prevalence of physician diagnosed asthma, other prevalence figures are also obviously, more or less, confounded by this selection bias. As this is true for all epidemiological studies that include data for bronchial challenge tests, the argument is understandable, but not crucial if recognized.

Another type selection bias results from diversity in anthropometrical data of the individuals who are included in a BHR study cohort. A third type of bias considers the annual physiological decrement in $FEV_1$ that excludes a certain proportion of subjects from BHR follow up studies, and this has not received much attention.

#### 6.2.1.3. Lung function data at baseline

Most of the BHR-studies reviewed for this thesis have only used the $FEV_1$ predicted values, and most of the $FEV_1$ predicted values have been in the normal range. However, the absolute baseline $FEV_1$ volumes in an individual level vary a lot, and based on results of the present study and others' (Ulrik 1993 & 1996, Longhini *et al.* 2004), may impact on the evaluation and interfere comparison of the results of the inducible bronchoconstriction between different cohorts.

The baseline $FEV_1$, i.e. airway calibre, has been discussed intermittently since the 1980s: Moreno *et al.* (1986) presented data of smooth muscle contraction determined by the variation of the calibre of the bronchus, further Bourbeau *et al.* (1993) studied the anatomical difference of trachea length and diameter in terms of deposition effects, and Brusasco & Pellegrino (2003) have published about factors modulating airway narrowing. Findings by de Marco *et al.* (1998) indicated a reduced risk for BHR with an increasing baseline $FEV_1$ [L]. In this Italian part of the ECRHS I study, risk for BHR was assessed by cut off levels $FEV_1$ 3.29 L and 4.08 L, which yielded OR 0.45 (95% CI) and OR 0.23 (95% CI 0.1-0.4), respectively, for BHR. Functional imaging of the airways and BHR-studies in a longitunal setting may provide more information in the future.

### 6.2.2. STATISTICAL ANALYSES IN EPIDEMIOLOGICAL BHR STUDIES

#### 6.2.2.1. Continous versus dichotomous variables

If subjects are excluded from epidemiological BHR studies because of a low FEV1-value, had been included in the statistical analyses by recoding them as the most severely affected individuals, the results of the logistic regression analysis would have benefited from a bigger sample size. This would have increased the representativeness of the results, and defined associations and risk factors in a more comprehensive way.

This is true in the longitudinal studies as well, in which subjects are excluded due to annual physiological decrement in $FEV_1$. In the case of the presented FinEsS-Helsinki incident asthma and BHR follow up study, for example, six subjects had their baseline $FEV_1 \leq 1.75$L in 2001-2003, which indicates that with an annual 25ml physiological decrement in $FEV_1$ these subjects would be excluded from 10-year follow up measurements because of a low baseline $FEV_1$ value. All these six subjects were women, thus causing a drop out in the proportion of women. This might subsequently confound some of gender dependent associations.

The logistic regression analysis enables testing of validated cut off levels, and provides opportunities to investigate new ones as well, as done in the presented

studies (Hosmer & Lemeshow 2000). By categorization of a variable, the problem of defining of those subjects who do not present BHR, is overcome.

#### *6.2.2.2. Dose response slope (DRS) and dose response ratio (DRR)*

Dose response slope (DRS) and dose response ratio (DRR) have been suggested in epidemiological studies, where the majority of those studied do not represent BHR (Sterk *et al.* 1985, O'Connor 1987, Peat *et al.* 1994). It is common to use a constant in the calculation of DRS that might cause some bias for the BHR. As published by Curjuric *et al.* (2011), a change in the slope might present a more informative, realiable, and suitable method for presenting data in an epidemiological setting.

#### 6.2.2.3. Different $FEV_1$ response limits

In general, different $FEV_1$ response limits can be used in bronchial challenge tests, but the use of higher $FEV_1$ response limits necessitates the use of higher doses of constrictors. In a population with mild airway responsiveness, lower $FEV_1$ cut-off levels have been suggested (Michoud *et al.* 1982, Neijens *et al.* 1982, Popa & Singleton 1988, Fardon *et al.* 2004). Investigation of an absolute $FEV_1$ decrement gives a real picture of the change in the induced flow limitation, such as the exercise induced bronchoconstriction (EIB), and might be useful in evaluation of sub-maximal responses of airway obstruction and perception of dyspnea.

#### *6.2.2.4. $PD_{15}$ / $PD_{20}$ $FEV_1$*

Methods based on the $PD_{15/20}FEV_1$ values provide quantative data of BHR. New research data as defined in PD-values are implicated to the clinical praxis without conversions, and the $PD_{15/20}FEV_1$ values can be individually followed in a longitudinal setting in taking care of a patient.

Intra-individual variation in PD values of histamine vs. methacholine, as a limitation, could be explained by differences in the position and form of the dose-response curves of $FEV_1$ to histamine and methacholine. The curves are typically more sigmoid than linear, which hinders the procurement of repeatable PD results for less hyperreactive subjects (Woolcock *et al.* 1984, Sterk *et al.* 1985, Lougheed *et al.*1993), but presents a high reproducibility in asthmatic subjects (Chinn & Schouten 2005).

### 6.2.3. BHR IN A LONGITUDINAL SETTING

#### *6.2.3.1. Baseline measurements of BHR in childhood and follow up*

BHR is commonly assessed in addition to replies of respiratory symptoms in defining asthma or respiratory illness (Rasmussen *et al.* 1999, Kotaniemi-Syrjänen *et al.* 2007, Meren *et al.* 2005, Chinn *et al.* 2007). In children 5-10 years of age, $FEV_1$ measurements during a challenge test could be applied (Malmberg *et al.* 2001). Spirometry could be recommened from 7-9 years of age onwards, and it is propably the most commonly used measurement of ventilation from then on (Escobar & Carver 2011). However, representativiness of the spirometry measurements should be carefully evaluated (Malmberg *et al.* 2001), and measurements that do not fulfill the ATS/ ERS criteria (Pellegrino *et al.* 2005) for repeatability by a single measure or by the three comparable curves due to a lack of co-operation, should absolutely be excluded from the analysis. The baseline measurements are the most critical in the longitudinal setting.

New pulmonary lung function techniques, such as impulse oschillometry (IOS), give comparable results to the spirometric values (Goldman *et al.* 2002, Houghton *et al.* 2004, Evans *et al.* 2005), thus could be considered in younger and less co-operative individuals (Escobar & Carver 2011, Shi *et al.* 2012).

#### *6.2.3.2. BHR and incident asthma in longitudinal studies*

There is no golden standard to measure and define the abnormally increased BHR, which make the comparison of different epidemiogical studies more or less unreliable in terms of incidence of asthma. However, an equal criterion for the population at risk enables the assessment and comparison of new asthma cases between different studies.

The ERS and ATS task forces could publish clinically validated BHR cut off levels in terms of follow-up instructions for abnormally increased BHR. Evidence of BHR as an independent determinant for incident respiratory symptoms, lung function decrement, and incident asthma, exist based on reports from the SAPALDIA-, ECRHS I and II – studies (Jansen *et al.* 1997, de Marco *et al.* 1998, Janson *et al.* 2001, Schwartz *et al.* 2002, Chinn *et al.* 1997 & 2007, Brutsche *et al.* 2006, Curjuric *et al.* 2011).

# 7 SUMMARY OF RESULTS AND CONCLUSIONS

## THE MAIN RESULTS OF THE THESIS

1. **H**istamine $PD_{15}FEV_1$ and methacholine $PD_{20}FEV_1$ methods have a good agreement in assessment of BHR severity among subjects without physician diagnosed asthma or chronic bronchitis.

2. **T**he prevalence of BHR in subjects without physician diagnosed asthma assessed by the histamine test ($PD_{15}FEV_1 \leq 1.6$ mg) was 28%, and by the methacholine test ($PD_{20}FEV_1 \leq 2.6$ mg) was 20%, in Kemi.

3. **P**revalence of BHR defined as histamine $PD_{15}FEV_1 \leq 1.6$ mg was 21% in the general adult population in Helsinki. Prevalence of moderate or severe BHR (BHRms) defined as histamine $PD_{15}FEV_1 \leq 0.4$ mg was 6% in the Helsinki adult population.

4. **T**he main risk factor for BHR was decreased $FEV_1$; showing a five-fold risk for BHR and a 14-fold risk for BHRms.

   **D**ecreased $MEF_{50}$ increased the risk for BHR and BHRms by 7- to 17- fold, respectively.

5. **O**f the subjects with BHR 69 % were ever smokers, and 32% current smokers. For BHRms the percentages were 83% and 56%, respectively.

   **B**HR was dose dependently associated with pack years of smoking.

   **E**xposure to environmental tobacco smoke (ETS) at work was a risk factor for BHR.

6. **A**llergic constitution was not significantly associated with BHR in the general adult population. Atopy combined with obstruction yielded a six-fold risk for BHR.

   **S**ubjects tested during pollen season presented a higher risk for BHR than subjects tested outside the season.

**M**ultisensitization (positive skin prick test at least for six allergens) yielded a four-fold risk for BHRms.

**O**besity (BMI>30 kg/m$^2$) was not significantly associated with BHR.

7. **E**xhaled nitric oxide (FENO) leveled the BHR severity only among the non-smokers.

8. **T**he incidence of asthma was 3.7/1000 persons/year.

   **R**emission of asthma was 17% during the follow up of 11 years.

   **A**llergic rhinitis doubled the risk for incident asthma, whereas living in a farm below five years of age reduced the risk for incident rhinoconjunctivitis.

## CONCLUSIONS

BHR carries a remarkable role in the dynamic changes of lung function in the general adult population in Helsinki. One in five of the studied subjects presented BHR, and among ever smokers BHRms comprised in a four-folded magnitude. The findings suggest that the occurence of non-specifc BHR assessed by histamine is associated with several environmental and individual risk factors affecting the smooth muscle response in the airway.

Results of the present studies indicate that decreased ventilatory function, due to large or small airway obstruction, is the most essential determinant for BHR. This is probably due to the disturbed airway function and heterogeneity in ventilation typically found in asthmatic conditions or in chronic obstructive pulmonary disease. The correlation of BHR to $MEF_{50}$ supports the view of using BHR in the evaluation of small airway disease.

The results presented here revealed that smoking was an independent risk factor for BHR and BHRms in a dose-dependent manner. Of the ever smokers, the majority had started to smoke before the age of 20 years. Exposure to tobacco smoke in assessment of increased BHR in adulthood should be notified in planning anti-smoking actions worldwide.

Allergic consititution by itself was not a significant determinat of BHR in the adult general population. Our results are in line with the point of view that non-allergic conditions of the united airways, such as rhinits and remodelling changes of the airway epithelium, play an increasing role in respiratory diseases and symptoms in adulthood as recently reported by others. The determinants of BHR show a different pattern of risk factors with different ages.

Multiple positive reactions in the skin prick tests (multisensitization) increased the risk for BHRms, which is probably linked to eosinophilic inflammation in the airways typically seen in the corticosteroid-dependent eosinophilic asthma. The correlation of increased FENO and BHR among non-smokers only, reported in this study to date, supports this deduction.

By a direct dosimetric method, BHR could be objectively measured. BHR should be evaluated in the assessment of respiratory symptoms if spirometry is normal as stated in the asthma guidelines (GINA, the Finnish Asthma Guideline). These results indicate that ever smokers and subjects with multisensitization are easily recommended for BHR testing. Allergic rhinoconjunctivitis was not associated with increased BHR, although ARC resulted as one of the main risk factors for adult onset asthma during an 11-year follow up.

Finally, methodological aspects such as the assessment of low $FEV_1$ value in terms of BHR severity, gender, and obesity could be considerated for further general population studies of BHR. From the clinical point of view, BHR targeted treatment trials, could provide a new approach for the evaluation of the effectiveness of new anti-inflammatory and anti-cholinergic drugs in chronic respiratory symptoms and diseases. New technology will enable the use of risk profile calculations for respiratory events, and such models could provide calculation frames for evaluation of cost effectiveness of BHR's position in evalution and tretament of respiratory symptoms and diseases.

The issue of BHR is complex. Findings of this thesis suggest that quantitative assessment of BHR by different cut off levels provides a tool for characterization of phenotypes of airway disorders in epidemiological studies. It is challenge for all physicians to implicate all of the available pathophysiological and epidemiological data in our clinical work.

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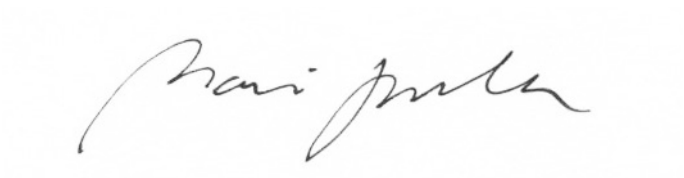
Espoo, August 2012



Maria Juusela

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# APPENDICES

**APPENDIX I** **FinEsS-tutkimus 2007**
**Nimi:**

Merkitse seuraavissa kysymyksissä rasti mielestäsi sopivien vaihtoehtojen kohdalle!
(siis jokaiseen kysymysvaihtoehtoon joko **kyllä** tai **ei/en tiedä**)

| | **Kyllä** | **Ei / En tiedä** |
|---|---|---|
| 1. Onko jollakin **vanhemmistasi** tai **sisaruksistasi** ? | | |
| a) astmaa | ( ) | ( ) |
| b) allergista nuhaa (esim. heinänuhaa) tai allergista silmävaivaa | ( ) | ( ) |
| c) kroonista keuhkoputkentulehdusta, keuhkoahtaumatautia tai keuhkonlaajentumaa | ( ) | ( ) |
| 2. Onko **Sinulla** nyt tai onko **Sinulla** ollut aiemmin ? | | |
| a) astmaa | ( ) | ( ) |
| b) allergista nuhaa (esim. heinänuhaa) tai allergista silmävaivaa | ( ) | ( ) |
| c) kroonista keuhkoputkentulehdusta, keuhkoahtaumatautia tai keuhkonlaajentumaa | ( ) | ( ) |
| d) muu keuhko- tai hengityselinsairaus, mikä ? | ( ) | ( ) |
| kirjoita vastaus tähän: _____ | | |
| 3. Onko lääkäri todennut Sinulla olevan astmaa ? | ( ) | ( ) |
| **Jos vastasit kyllä:** Minkä ikäinen olit kun astma todettiin? _____vuotta | | |
| 4. Onko lääkäri todennut Sinulla olevan kroonista (pitkäaikaista) keuhkoputkentulehdusta, keuhkoahtaumatautia tai keuhkonlaajentumaa? | ( ) | ( ) |
| 5. Käytätkö astmalääkkeitä ? Mitä lääkettä: _____ | ( ) | ( ) |
| 6. Onko Sinulla ollut astmaoireita nykyisin tai viimeisten 10 vuoden aikana ? (ajoittain hengenahdistusta tai hengenahdistuskohtauksia, samanaikaisesti voi olla yskää tai hengityksen vinkumista) | ( ) | ( ) |
| **Jos vastasit kyllä:** | | |
| a) Onko sinulla ollut astmaoireita viimeisten 12 kuukauden aikana ? | ( ) | ( ) |
| 7. Onko Sinulla ollut viime vuosina pitkäaikaista yskää ? | ( ) | ( ) |
| 8. Nouseeko yskiessä keuhkoistasi yleensä limaa tai onko keuhkoissa limaa, jota on vaikea saada irtoamaan ? | ( ) | ( ) |
| **Jos vastasit kyllä:** | | |
| a) onko limaa noussut useimpina päivinä vähintään kolmen peräkkäisen kuukauden aikana? | ( ) | ( ) |
| b) onko tällaisia vähintään kolmen kuukauden limannousujaksoja ollut vähintään kahtena peräkkäisenä vuonna? | ( ) | ( ) |
| 9. Onko tavallista, että keuhkoissa hengityksesi rahisee äänekkäästi tai vinkuu ? | ( ) | ( ) |
| 10. Onko sinulla ollut hengityksen vinkunaa kertaakaan viimeisten 12 kuukauden aikana ? | ( ) | ( ) |
| **Jos vastasit kyllä:** | | |
| a) oliko Sinulla ollenkaan hengenahdistusta vinkunan yhteydessä ? | ( ) | ( ) |
| b) oliko Sinulla hengityksen vinkunaa vaikka et ollut flunssassa ? | ( ) | ( ) |
| 11. Oletko viimeisten 12 kuukauden aikana herätessäsi kertaakaan kokenut hengityksesi olevan tukossa? | ( ) | ( ) |
| 12. Hengästytkö tai joudutko hengästymisen vuoksi kävelemään hitaammin kuin muut, kun kävelet ikäistesi henkilöiden kanssa tasamaalla normaalivauhdilla? | ( ) | ( ) |

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| | Kyllä | Ei / En tiedä |
|---|---|---|
| 13. Saatko hengenahdistusta, hengityksen vinkunaa tai vaikeita yskänpuuskia? | | |
| a) rasituksessa | ( ) | ( ) |
| b) kylmässä | ( ) | ( ) |
| c) pakkasessa rasituksen yhteydessä | ( ) | ( ) |
| d) pölyisissä olosuhteissa | ( ) | ( ) |
| e) tupakansavusta | ( ) | ( ) |
| f) autojen pakokaasuista | ( ) | ( ) |
| g) voimakkaista tuoksuista (esim. deodorantit, mausteet, painomuste, puhdistusaineet, kukkien tuoksut tms.) | ( ) | ( ) |
| h) kasvien tai puiden siitepölyistä | ( ) | ( ) |
| i) ollessasi tekemisissä eläinten kanssa | ( ) | ( ) |
| 14. Poltatko savukkeita, sätkää, piippua tai sikareita? (vastaa kyllä vaikka olisitkin lopettanut viimeisten 12 kuukauden aikana) | ( ) | ( ) |
| **Jos vastasit kyllä:** | | |
| a) Kuinka monta savuketta, sätkää, sikaria tai piipullista olet | | |
| polttanut ***keskimäärin vuorokaudessa*** ? a) vähemmän kuin 5 kpl | ( ) | ( ) |
| b) 5-14 kpl | ( ) | ( ) |
| c) 15-24 kpl | ( ) | ( ) |
| d) vähintään 25 kpl | ( ) | ( ) |
| **Jos vastasit ei:** | | |
| b) Oletko aiemmin ollut tupakoitsija, mutta lopetit yli vuosi sitten? | ( ) | ( ) |

15. Jos olet tai olet ollut tupakoitsija minkä ikäisenä aloitit? _____vuotiaana

a) Minkä ikäisenä lopetit? _____vuotiaana

16. Missä ammatissa olet pääasiassa toiminut? Kirjoita vastaus tähän: _____

a) minä vuosina olet ollut tässä työssä? vuodesta _____ vuoteen _____

| | | |
|---|---|---|
| 17. Onko Sinulla nykyisin muu työ tai tehtävä (muu ammatti, opiskelu, työtön, kotityö, eläke, sairauseläke tms.)? | ( ) | ( ) |

jos on niin mikä?, kirjoita vastaus tähän: _____

a) montako vuotta olet yhteensä ollut tässä työssä, eläkkeellä, tms.: _____vuotta

| | | |
|---|---|---|
| 18. Onko työympäristössäsi nyt tai onko työympäristössäsi ollut aikaisemmin paljon pölyä, kaasuja tai savua? | ( ) | ( ) |

19. Mikä on pituutesi ja painosi?: _____cm _____kg

20. Kuinka paljon painosi on muuttunut kymmenen vuoden aikana?

a) noussut_____ kg / laskenut _____kg

| | | |
|---|---|---|
| 21. Asuitko maaseudulla (ei kaupungissa eikä taajamassa) ensimmäisten 5 elinvuotesi aikana? | ( ) | ( ) |
| a) Asuitko maatilalla ensimmäisten 5 elinvuotesi aikana? | ( ) | ( ) |
| 22. Onko terveydentilassasi tapahtunut jokin merkittävä muutos viimeisen 10 vuoden aikana? | ( ) | ( ) |

Tarkenna jos vastasit kyllä:

**Päiväys:** **Puhelinnumerosi:**

**Allekirjoitus:**

Vastaukset kerätään tutkimusrekisteriin Filha Ry, Sibeliuksenkatu 11 A1, 00250 Helsinki, 09-45421265
ja Keuhkosairauksien tutkimusyksikköMeilahden sairaala, HYKS, Haartmaninkatu 4, PL 340, 00029 HUS, p 09-4711

**APPENDIX II** **FinEsS-studien 2007**

**Namn:**

Besvara frågorna genom att kryssa för ett lämpligt alternativ.
(besvara varje fråga antingen med **JA** eller **NEJ / VET EJ)**

| | **JA** | **NEJ / VET EJ** |
|---|---|---|
| **1.** Är det någon av Dina **föräldrar** eller **syskon** som har? | | |
| a) astma | ( ) | ( ) |
| b) allergisk snuva (t.ex hösnuva) eller allergiska ögonbesvär | ( ) | ( ) |
| c) kronisk luftrörskatarr, kronisk obstruktiv lungsjukdom eller emfysem | ( ) | ( ) |
| **2.** Har **Du** nu eller har **Du** tidigare haft? | | |
| a) astma | ( ) | ( ) |
| b) allergisk snuva (t.ex hösnuva) eller allergiska ögonbesvär | ( ) | ( ) |
| c) kronisk luftrörskatarr, kronisk obstruktiv lungsjukdom eller emfysem | ( ) | ( ) |
| d) annan lung- eller luftvägssjukdom, vilken? _____ | | |
| **3**. Har Du av läkare fått diagnosen astma? | ( ) | ( ) |
| **Om Du svarade ja:** Hur gammal var Du då astman konstaterades? _____ år | | |
| **4**. Har Du av läkare fått diagnosen kronisk (långvarig) luftrörskatarr (bronkit), kronisk obstruktiv lungsjukdom eller emfysem? | ( ) | ( ) |
| **5.** Använder Du astmamediciner? Vilka läkemedel? _____ | ( ) | ( ) |
| **6.** Har Du nu eller har Du under de senaste 10 åren haft astmasymptom? (periodvis andnöd eller andnödsanfall, besvären kan uppträda med samtidig hosta eller pip i bröstet) | ( ) | ( ) |
| **Om Du svarade ja:** a) Har Du haft astmasymptom under de senaste 12 månaderna? | ( ) | ( ) |
| **7.** Har Du under de senaste åren haft långvarig hosta? | ( ) | ( ) |
| **8.** Brukar Du hosta upp slem eller har Du slem i lungorna, vilket är svårt att få upp? | ( ) | ( ) |
| **Om Du svarade ja:** a) Har Du fått upp slem de flesta dagar under en period som varat minst 3 månader | ( ) | ( ) |
| b) Har Du haft sådana 3 månaders perioder minst 2 år i rad? | ( ) | ( ) |
| **9.** Brukar Dina lungor rossla hörbart eller brukar din andning pipa? | ( ) | ( ) |
| **10.** Har Du haft pip i bröstet vid något tillfälle under de senaste 12 månaderna? | ( ) | ( ) |
| **Om Du svarade ja:** a) Har Du överhuvudtaget varit det minsta andfådd när Du haft pip i bröstet? | ( ) | ( ) |
| b) Har Du haft pip i bröstet utan att samtidigt vara förkyld? | ( ) | ( ) |
| **11.** Har Du vaknat med trånghetskänsla i bröstet vid något tillfälle under de senaste12 månaderna? | ( ) | ( ) |
| **12.** Blir Du andfådd eller måste Du gå långsammare än jämnåriga på plan mark i normal takt på grund av andfåddhet? | ( ) | ( ) |

b) i kyla ( ) ( )

c) vid ansträngning utomhus i kallt väder ( ) ( )

d) i dammiga miljöer ( ) ( )

e) av tobaksrök ( ) ( )

f) av bilavgaser ( ) ( )

g) av starka dofter ( ) ( )

(deodoranter, kryddor, trycksvärta, rengöringsmedel, blomsterdoft osv)

h) av pollen från växter eller träd ( ) ( )

i) i kontakt med djur ( ) ( )

**14.** Röker Du cigaretter, handrullade cigaretter, pipa eller cigarrer? ( ) ( )
(svara ja även ifall Du slutat röka inom de senaste 12 månaderna)

**Om Du svarade ja:**

a) Hur många cigaretter, handrullade cigaretter, cigarrer eller pipstopp ha Du rökt i ***medeltal per dygn***

a) mindre än 5 st ( )

b) 5 – 14 st ( )

c) 15 –24 st ( )

d) minst 25 st ( )

**Om Du svarade nej:**

b) Har Du tidigare varit rökare men slutat för mer än 1 år sedan? ( ) ( )

**15.** Om Du är eller har varit rökare, i vilken ålder började Du röka? Som _____-åring

a) I vilken ålder slutade Du röka? Som _____-åring

**16.** Vilket har varit Ditt huvudsakliga yrke? _____

a) Hur många år har Du varit i detta yrke? Från år _____ till år _____

**17.** Är Du nu i någon annan verksamhet / sysselsättning ( ) ( )
(annat yrke, studier, arbetslös, hemarbete, pensionär, sjukpensionär o. dyl.)?

**Om Du svarade ja: vilket?** _____

a) Hur många år har Du sammalagt varit i denna verksamhet, pensionär el. dyl.

_____år

**18.** Finns det nu eller har det tidigare funnits mycket damm, gaser eller rök i Din arbetsmiljö? ( ) ( )

**19.** Vad är Din längd och Din vikt? _____cm _____kg

**20.** Hur mycket har Din vikt förändrats de senaste 10 åren?

a) ökat _____kg / minskat _____kg

**21.** Bodde Du på landsbygden (inte i stad eller tätort) under Dina 5 första levnadsår? ( ) ( )

a) Bodde du på en lantgård under Dina 5 första levnadsår? ( ) ( )

**22.** Har det skett någon väsentlig förändring i Ditt hälsotillstånd under de senaste 10 åren? ( ) ( )

Precisera om Du svarade ja:_____

**Datum:**_____ **Ditt telefonnummer**:_____

**Underskrift**:_____

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Svaren samlas i forskningsregister vid Filha ry, Sibeliusgatan 11 A1, 00250 Helsinki, puh 09-45421265, och Lungmedicinska Forskningsenhetens, Meilans sjukhus, HUCS, Haartmansgatan 4, PB 340, 00029 HNS, tel. 09-4711

## APPENDIX III

**FinEsS tutkimus** **Hengityssairauksien haastattelu** (2001-01-26 amended version)
**Helsinki**

haastattelija _____ tutkimuspäivä _____

tutkittavan
sukunimi _____

etunimet _____

osoite _____

postinumero ja -toimipaikka _____

puhelinnumerot: koti _____ työ _____

**1.** syntymäaika ( tai koko henkilötunnus )_____

**2.** Finess numero _____

**3.** rotu
- 1. valkoinen rotu ( )
- 2. musta rotu ( )
- 3. keltainen rotu ( )
- 4. muu ( )

**4.** sukupuoli
- mies ( )
- nainen ( )

**5.** otos
- satunnaisotos ( )
- suunnattu otos ( )

**6.** koodit:
- maa ____
- keskus ____
- alue ____
- kansallisuus ____

**7.** väestötiheyden luokitus (ei käytössä Helsingissä)





**Yskä ja limannousu**

**8.** Onko Sinulla ollut viime vuosina pitkäaikaista yskää? ei ( )
kyllä ( )

**9.** Yskitkö tai köhitkö yleensä aamuisin? ei ( )
kyllä ( )

vastaa kysymykseen 10 vain jos vastasit **kyllä** kysymykseen 9.

10. Onko Sinulla tällaista yskää tai köhää aamuisin ei ( )
useimpina viikon päivinä yli kahden viikon jaksoissa? kyllä ( )

**11.** Yskitkö tai köhitkö muuhun aikaan päivästä tai öisin? ei ( )
kyllä ( )

vastaa kysymykseen 12 vain jos vastasit **kyllä** kysymykseen 11.

12. Onko Sinulla tällaista yskää tai köhää useimpina ei ( )
viikon päivinä yli kahden viikon jaksoissa? kyllä ( )

vastaa kysymykseen 13 vain jos vastasit **kyllä** kysymykseen 10 tai 12:

13. Onko Sinulla tällaista yskää... - harvakseen silloin tällöin? ( )
- yleensä / tavallisesti talvella? ( )
- pitkin vuotta jaksoittain tai kaikkina päivinä? ( )

**14.** Nouseeko yskiessä tai köhiessä keuhkoistasi yleensä limaa? ei ( )
silloin tällöin ( )
usein ( )

**15.** Onko Sinulla keuhkoissa limaa, jota on vaikea saada ei ( )
irtoamaan? kyllä ( )

**16.** Esiintyykö Sinulla yskäjaksoja, jolloin useimpina päivinä yskiessä tai köhiessä
keuhkoistasi yleensä nousee limaa tai onko keuhkoissa ei, tai <3kk/vuosi ( )
limaa, jota on vaikea saada irtoamaan? vähintään 3kk/vuosi ( )
kahtena peräkkäisenä vuonna vähintään 3kk/vuosi ( )

vastaa kysymykseen 17 vain jos rastitit kysymyksen 16 viimeisen vaihtoehdon:

17. Kuinka monen vuoden ajan? _____ vuotta

vastaa kysymykseen 18 vain jos rastitit kysymyksen 16 ensimmäisen vaihtoehdon:

18. Onko Sinulla koskaan ollut pitkäaikaista yskää tai limannousua ei ( )
keuhkoista pitkäkestoisina jaksoina? kyllä ( )

**Hengityksen vinkunat**

**19.** Esiintyykö Sinulla ajoittain vinkuvaa tai muutoin poikkeavan äänekästä hengitystä? ei ( ) kyllä ( )

**20.** Oletko koskaan havainnut hengityksesi vinkuvan ( keuhkoissa ) ? ei ( ) kyllä ( )

**21.** Onko Sinulla ollut hengityksen vinkunaa kertaakaan viimeisten 12 kuukauden aikana? ei ( ) kyllä ( )

vastaa kysymyksiin 22-24 (25) vain jos vastasit **kyllä** kysymykseen 21

22. Onko Sinulla ollut vähäistäkään hengenahdistusta vinkunan yhteydessä? ei ( ) kyllä ( )

23. Onko Sinulla ollut hengityksen vinkunaa silloinkin kun et ole ollut flunssainen? ei ( ) kyllä ( )

24. Onko hengityksesi vinkuvaa tai poikkeavan äänekästä viikon useimpina päivinä? ei ( ) vain jaksoittain ( ) kyllä, pitkin vuotta ( )

mikäli vastasit: JAKSOITTAIN kysymykseen 24, merkitse minkä kuukausien aikana:

25. Kuukaudet jolloin hengityksen vinkunoita esiintyy:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tammi | | Helmi | | Maalis | | Huhti | | Touko | | Kesä | |
| Heinä | | Elo | | Syys | | Loka | | Marras | | Joulu | |

ei pysty nimeämään tiettyjä kuukausia ( )

**Hengenahdistus yleensä**

**26.** Onko Sinulla liikuntavaikeuksia muusta syystä kuin keuhko- tai sydänsairauden vuoksi? ei ( ) kyllä ( )

vastaa kysymykseen 27 vain jos vastasit **kyllä** kysymykseen 26:

27. Mistä syystä? aivoverenkiertosairaus ( ) lihassairaus ( ) raajojen liikkuvuus rajoittunut ( ) muut syyt ( )



**28.** Oletko pyörätuolin käyttäjä? ei ( ) kyllä ( )

**29.** Esiintyykö Sinulla koskaan hengenahdistusta tai hengitysvaikeutta? ei ( ) kyllä ( )

vastaa kysymykseen 30 vain jos vastasit **kyllä** kysymykseen 29:

30. Esiintyykö Sinulla tällaista hengenahdistusta tai hengitysvaikeutta?
jatkuvasti, hengitys ei koskaan ole täysin kunnossa ( )
toistuvasti, mutta hengitys palautuu aina tavalliseksi ( )
vain harvoin ( )

**31.** Esiintyykö Sinulla hengenahdistusta tai hengitysvaikeutta kiirehtiessäsi tasamaalla tai kun kävelet ylös loivaa mäkeä tai noustessasi rappuja yhden kerrosvälin omalla vauhdillasi? ei ( ) kyllä ( )

vastaa kysymyksiin 32-34 vain jos vastasit **kyllä** kysymykseen 31:

32. Hengästytkö, kun kävelet tasamaalla ikäistesi henkilöiden kanssa? ei ( ) kyllä ( )

33. Joudutko pysähtymään hengähtääksesi välillä kun kävelet omalla vauhdillasi tasamaalla? ei ( ) kyllä ( )

34. Hengästytkö pukeutuessasi tai riisuutuessasi? ei ( ) kyllä ( )

**Kohtauksittainen hengenahdistus ja ahtauden tuntu hengityksessä**

**35.** Onko Sinulla koskaan ollut hengenahdistuskohtauksia tai ajoittain esiintyvää poikkeavan tuntuista hengästymistä? ei ( ) kyllä ( )

**36.** Onko Sinulla ollut yhtään hengenahdistuskohtausta tai hengästymiskohtausta viimeisten 12 kuukauden aikana? ei ( ) kyllä ( )

vastaa kysymykseen 37 vain jos vastasit **kyllä** kysymykseen 35 tai 36:

37. Onko hengityksesi normaalia hengenahdistus-kohtausten välillä tai ennen kohtauksen alkua? ei ( ) kyllä ( )

**38.** Onko Sinulla ollut koskaan hengenahdistuskohtausta ja samanaikaisesti hengityksen vinkunaa? ei ( ) kyllä ( )

vastaa kysymykseen 39 ja 40 vain jos vastasit **kyllä** kysymykseen 38:

39. Onko Sinulla ollut hengenahdistuskohtausta ja samalla hengityksen vinkunaa viimeisten 12 kuukauden aikana? ei ( ) kyllä ( )

40. Minkä ikäisenä Sinulla oli ensimmäinen hengenahdistuskohtaus samalla esiintyneen hengityksen vinkunan kanssa? ikä: _____v

**41.** Oletko koskaan herännyt yöllä tai varhain aamulla hengenahdistus-kohtauksen ja samanaikaisen hengityksen vinkunan vuoksi? ei ( ) kyllä ( )

vastaa kysymykseen 42 vain jos vastasit **kyllä** kysymykseen 41:

42. Onko näin käynyt viimeisten 12 kuukauden aikana? ei ( ) kyllä ( )

vastaa kysymykseen 43 vain jos vastasit **kyllä** kysymyksiin 36,39 tai 42:

**43.** Kuinka monta hengenahdistuskohtausta, vinkunan kanssa tai ilman, Sinulla on ollut viimeisten 12 kuukauden aikana?

a) ei yhtään ( )
b) ehkä kerran ( )
c) kahdesta viiteen kertaan ( )
d) yli viisi kertaa, mutta ei useimpina kuukausina ( )
e) useimpina kuukausina mutta ei useimpina viikkoina ( )
f) useimpina viikkoina mutta ei useimpina päivinä ( )
g) useimpina päivinä ( )
h) jaksoittain useimpina viikkoina ( )
i) jaksoittain useimpina päivinä ( )

**44.** Onko Sinulla koskaan ollut ahtauden tunnetta rinnassa? ei ( ) kyllä ( )

vastaa kysymykseen 45 vain jos vastasit **kyllä** kysymykseen 44:

45. Onko tällaista tuntemusta ollut viimeisten 12 kk aikana? ei ( ) kyllä ( )

**46.** Oletko koskaan herätessäsi kokenut ahtauden tunnetta rinnassa? ei ( ) kyllä ( )

vastaa kysymykseen 47 vain jos vastasit **kyllä** kysymykseen 46:

47. Onko näin käynyt viimeisten 12 kk aikana? ei ( ) kyllä ( )





**Tekijöitä, jotka aiheuttavat Sinulle hengityksen vinkunaa tai hengenahdistuskohtauksia, yskän kera tai ilman yskää**

**48.** Karvaiset eläimet, esim.; koira, kissa, lehmä, hevonen, kani jne… ei ( ) kyllä ( )

**49.** Siitepölyaltistus, esim.; lehdet, ruoho, ulkokukat ei ( ) kyllä ( )

**50.** Homeen haju tai homealtistus ei ( ) kyllä ( )

**51.** Tupakan savu tai haju ei ( ) kyllä ( )

**52.** Pölyisissä olosuhteissa yleensä ei ( ) kyllä ( )

**53.** Voimakkaat tuoksut tai hajut (deodorantit, mausteet, painomuste, savukaasut, puhdistusaineet, kukkien tuoksut jne…) ei ( ) kyllä ( )

**54.** Autojen pakokaasut ei ( ) kyllä ( )

**55.** Muut ilmansaasteet ei ( ) kyllä ( )

**56.** Hengitystieinfektiot, nuhakuumeet ei ( ) kyllä ( )

**57.** Lääkkeet ei ( ) kyllä ( )

Jos **kyllä**, mitkä lääkkeet?

_____

_____

**58.** Ruoka, esim.: kala, äyriäiset, pähkinät, siemenhedelmät ei ( ) kyllä ( )

Jos **kyllä**, mikä ruoka?

_____

_____

**59.** Psyykkiset tekijät tai stressi? ei ( )
kyllä ( )

**60.** Kylmä ilma? ei ( )
kyllä ( )

**61.** Muu säätila kuten kostea ilma, tuulinen, sumuinen tai lämmin ilma? ei ( )
kyllä ( )

**62.** Tuleeko Sinulle fyysisen rasituksen jälkeen välittömästi tai muutaman minuutin kuluttua hengenahdistusta ja hengityksen vinkunaa? ei ( )
kyllä ( )

**63.** Tuleeko Sinulle fyysisen rasituksen aikana hengenahdistusta ja hengityksen vinkunaa? ei ( )
kyllä ( )

**64.** Onko Sinulla ollut koskaan hengenahdistuskohtauksia ja hengityksen vinkunoita tai astman oireita työpaikallasi? ei ( )
kyllä ( )

vastaa kysymykseen 65 vain jos vastasit **kyllä** kysymykseen 64:

65. Onko näin käynyt viimeisten 12 kk aikana? ei ( )
kyllä ( )

**66.** Saatko hengenahdistusta ja hengityksen vinkunaa muista syistä kuin yllä mainittu? ei ( )
kyllä ( )

jos **kyllä;** täsmennä: _____

**Astma ja krooninen keuhkoputken tulehdus ( krooninen bronkiitti )**

**67.** Onko Sinulla koskaan ollut astmaa? ei ( )
kyllä ( )
en tiedä ( )

**68.** Onko lääkäri todennut Sinulla olevan astmaa? ei ( )
kyllä ( )
en tiedä ( )

**69.** Onko Sinulla esiintynyt astmaa lapsuuden aikana tai hengityksen vinkunoita varhaisessa lapsuudessa? ei ( )
kyllä ( )
en tiedä ( )

**70.** Käytätkö tai oletko aiemmin käyttänyt astmalääkkeitä säännöllisesti tai tarvittaessa? ei ( )
kyllä ( )



**71.** Onko lääkäri määrännyt Sinulle mitään astmalääkettä? ei ( )
kyllä ( )

vastaa kysymyksiin 72-76 vain jos vastasit **kyllä** johonkin kysymyksistä 67-71:

72. Minkä ikäinen olit kun lääkäri kertoi Sinulla olevan astmaa tai määräsi Sinulle astmalääkettä? Ikä _____ v

73. Minkä ikäinen olit kun Sinulla oli ensimmäinen astmakohtaus tai astmaan liittyvä tapahtuma tai oireinen astmajakso Ikä _____ v

74. Minkä ikäinen olit kun Sinulla oli viimeisin astmakohtaus ? Ikä _____ v

75. Onko Sinulla ollut ylipäänsä mitään astmaoireita viimeisten 12 kuukauden aikana? ei ( )
kyllä ( )

76. Oletko käyttänyt mitään astmalääkkeitä viimeisten 12 kuukauden aikana? ei ( )
kyllä ( )

**77.** Onko lääkäri todennut Sinulla olevan kroonista keuhkoputken tulehdusta tai keuhkon laajentumaa? ei ( )
kyllä ( )
en tiedä ( )

**78.** Mikä on oma käsityksesi, onko Sinulla kroonista keuhkoputken tulehdusta? ei ( )
kyllä ( )
en tiedä ( )

**79.** Mikä on oma käsityksesi, onko Sinulla keuhkon laajentumatautia? ei ( )
kyllä ( )
en tiedä ( )

jos **kyllä vastauksia** kysymyksissä 76-79, täsmennä kysymykset 80-93, **mikäli** olet käyttänyt jotain kyseessä olevaa **lääkettä hengityssairauden vuoksi viimeisten 12 kk aikana:**

**80.** Hengitettävää, avaavaa (lyhytvaikutt. β-2agonistit) lääkettä? ei ( )
( aerosolina tai jauhemuodossa esim.: Ventoline, silloin tällöin ( )
Salbuvent, Buventol, Bricanyl, Berotec ) useimpina päivinä viikossa ( )

vastaa myös kysymykseen 81, jos edellisessä muu kuin **ei** vaihtoehto:

81. Milloin aloitit hengitettävän lyhytvaikutteisen avaavan ( β-2agonistit ) lääkkeen käytön? alle 1 v. sitten ( )
1-5 vuotta sitten ( )
yli 5vuotta sitten ( )

**82.** Hengitettävää kortisonivalmistetta? ei ( )
( Becotide, Beclomet, Pulmicort, silloin tällöin ( )
Flixotide, Cortivent,Seretide ) useimpina päivinä viikossa ( )

vastaa kysymyksiin 83 ja 84,jos edellä kysymyksessä 82 vastasit: silloin tällöin tai useimpina päivinä viikossa:

83. Milloin aloitit hengitettävän kortisonivalmisteen käytön? alle 1 vuosi sitten ( )
1-5 vuotta sitten ( )
yli 5 vuotta sitten ( )

84. Mikä on nykyinen annos? alle 200 mcg/vrk ( )
200-800 mcg/vrk ( )
> 800 mcg/vrk ( )

**85.** Antikolinergista lääkettä? ei ( )
( Atrovent, Atrovent comp, Ventox ) silloin tällöin ( )
useimpina päivinä viikossa ( )

**86.** Kromoglikaatteja tai nedokromiilia? ei ( )
(Lomudal, Tilade) silloin tällöin ( )
useimpina päivinä viikossa ( )

**87.** Hengitettävää pitkävaikutteista avaavaa ( β-2agonistit ) lääkettä? ei ( )
(Serevent, Foradil,Oxis,Seretide) silloin tällöin ( )
useimpina päivinä viikossa ( )

**88.** Avaavaa lääkettä ( β-2agonistit ) tablettimuodossa? ei ( )
( Ventoline, Salbuvent, Bricanyl ) silloin tällöin ( )
useimpina päivinä viikossa ( )

**89.** Teofylliinivalmistetta? ei ( )
( Euphyllin, Retafyllin, Theophyllin, silloin tällöin ( )
Aminocont, Theo-Dur, Nuelin ) useimpina päivinä viikossa ( )

**90.** Avaavaa lääkettä sumuttimella ( spiralla tms.)? ei ( )
silloin tällöin ( )
useimpina päivinä viikossa ( )

**91.** Kortisonivalmistetta tablettimuodossa? ei ( )
( esim. Medrol, Prednisolon ) silloin tällöin ( )
useimpina päivinä viikossa ( )
vain keuhkosairauden pahenemisvaiheiden yhteydessä ( )





jos **ei** kysymykseen 91, vastaa myös kysymykseen 92:

92. Oletko aiemmin käyttänyt kortisonivalmistetta tablettimuodossa? ei ( )
kyllä ( )

vastaa kysymykseen 93 vain jos vastasit **kyllä** kysymykseen 92:

93. Miksi lopetit kortisonitablettien käytön ? aloitin hengitettävän kortisonin ( )
sairaus lievittyi ( )
muu syy ( )
käytin vain keuhkosairauden pahenemisvaiheiden yhteydessä ( )

**Yskänlääkkeet ja limaairroittavat lääkkeet**

**94.** Käytätkö tai oletko aiemmin käyttänyt limaa irroittavaa tai yskänlääkettä enemmän kuin vain tilapäisesti flunssien aikana? ei ( )
kyllä ( )

vastaa kysymykseen 95 ja 96 vain jos vastasit **kyllä** kysymykseen 94:

95. N-Asetyylikysteiini valmistetta? ei ( )
( Mucomyst, Mucoporetta ) kyllä ( )

96. Muuta limaairroittavaa tai yskänlääkettä? ei ( )
kyllä ( )

**Lääkkeet nuhaoireisiin, nenäntukkoisuuteen ja silmäoireisiin**

**97.** Käytätkö tai oletko aiemmin käyttänyt pitkäaikaisesti tai toistuvasti lääkkettä nuhaan tai silmätulehdukseen? ei ( )
kyllä ( )

vastaa kysymyksiin 98 - 100 vain jos vastasit **kyllä** kysymykseen 97:

98. Antihistamiini tablettimuodossa ? ei ( )
( esim.: Teldanex, Semprex, Disofrol, Duact, Kestine Hismanal, Lunerin, Clarityn, Cirrus, Rinomar, Zyrtec ) kyllä ( )

99. Kortisoninenäsuihketta? ei ( )
( esim.: Beconase, Rhinocort, Flixonase, Lokilan Nasal Becotide Nasal, Beclonasal ) kyllä ( )

100. Muuta nenä- ja silmäoireiden lääkehoitoa ( esim. kromoglykaatti ) ei ( )
kyllä ( )

**Terveyspalveluiden tarve**

**101.** Onko Sinulla ylipäänsä mitään hengitysongelmia tai yskävaivoja tai limannousuongelmia? ei ( ) kyllä ( )

**102.** Oletko koskaan joutunut hakeutumaan lääkärille tai muuhun hoitoon hengenahdistuksen tai hengityksen vinkunan vuoksi? ei ( ) kyllä ( )

**103.** Oletko koskaan joutunut hakeutumaan lääkärille tai muuhun hoitoon pitkäaikaisen yskän tai limannousun vuoksi? ei ( ) kyllä ( )

vastaa kysymyksiin 104-115 jos vastasit **kyllä** yhteenkin kysymykseen 101-103:

104. Oletko joutunut hakeutumaan lääkärille hengitysoireiden, yskän tai limannousun vuoksi viimeisten 12kk aikana? ei ( ) kyllä ( )

vastaa kysymykseen 105 jos vastasit **kyllä** kysymykseen 104:

105. Kuinka monta kertaa olet joutunut hakeutumaan lääkärille hengitys-, yskä- tai limannousuongelmien vuoksi viimeisten 12 kk aikana? _____

106. Käytkö säännöllisesti lääkärillä hengitysongelmien vuoksi? ei ( ) kyllä ( )

107. Oletko hengitysvaivojen tai yskä- ja limannousuongelmien vuoksi käynyt keuhkolääkärillä tai allergologian erikoislääkärillä ? ei ( ) kyllä ( )

vastaa kysymykseen 108 vain jos vastasit **kyllä** kysymykseen 107:

108. Onko allergian selvittämiseksi tehty ihopistokoe? ( = ns. PRICK -testi ) ei ( ) kyllä ( )

vastaa kysymykseen 109 vain jos vastasit **kyllä** kysymykseen 108:

109. Löytyikö PRICK -testissä allergisuutta? ei ( ) kyllä ( )

110. Oletko koskaan joutunut päivystysluontoisesti hoitoon hengitysvaivojen vuoksi? ei ( ) kyllä ( )

111. Oletko ollut sairaalahoidossa hengitys-ongelmien vuoksi? ei ( ) kyllä ( )

112. Oletko tyytyväinen lääkäripalvelujen saatavuuteen kun olet tarvinnut apua hengitysvaivoihisi? ei ( ) kyllä ( ) en ole tarvinnut ( )



113. Koetko, että hengenahdistus tai hengästyneisyys tai hengityksen vinkuna häiritsee päivittäistä elämääsi? ei ( ) silloin tällöin ( ) usein ( )

114. Koetko, että yskä tai limannousu häiritsevät päivittäistä elämääsi? ei ( ) silloin tällöin ( ) usein ( )

115. Kuinka paljon mielestäsi hengitysoireesi haittaavat jokapäiväistä elämääsi? ei lainkaan ( ) vähäisesti ( ) ajoittain kohtalaisen runsaasti ( ) kohtalaisen runsaasti ( ) runsaasti ( )

**Muut kuin ahtauttavat keuhkosairaudet**

**116.** Onko Sinulla muuta keuhko- tai hengityselinsairautta kuin astma, krooninen bronkiitti tai emfyseema? ei ( ) kyllä ( )

vastaa kysymykseen 117. vain jos vastasit **kyllä** kysymykseen 116:

117. Mitä muuta sairautta:

1._____

2. _____

3. _____

**118.** Onko Sinulla ollut tuberkuloosi (TBC)? ei ( ) kyllä, keuhkotbc ( ) kyllä, muu tbc ( )

**119.** Onko Sinulla tai onko Sinulla ollut heinänuhaa tai allergista nuhaa tai silmätulehdusta? ei ( ) kyllä ( )

**120.** Vaivaako Sinua usein tukkoinen tai vuotava nenä? ei ( ) kyllä ( )

**121.** Onko Sinulla tai onko ollut nenäpolyyppeja? ei ( ) kyllä ( )

**122.** Onko Sinulla tai onko ollut (edes lapsuudessa) pitkäaikaista ihottumaa? ei ( ) kyllä ( )

**123.** Onko Sinulla tai onko ollut mitään tässä mainittua sydänvikaa tai sydänsairautta?
ei mitään ( )
angina pectoris ( )
sydäninfarkti ( )
sydämen vajaatoiminta ( )
rytmihäiriöitä ( )
muu sydänvika ( )

**124.** Käytätkö nykyisin sydänlääkitystä?
ei ( )
yksi lääke ( )
kaksi lääkettä ( )
kolme lääkettä ( )
neljä lääkettä tai enemmän ( )

**125.** Onko Sinulla tai onko ollut verenpainetautia? ei ( ) kyllä ( )

**126.** Onko Sinulla nykyään käytössä verenpainelääkitystä? ei ( ) kyllä ( )

126a. Käytätkö β-salpaajalääkettä? ei ( ) kyllä ( )

**127.** Onko Sinulla jotain muuta kroonista sairautta, jota ei ole edellä mainittu? ei ( ) kyllä ( )

127a. Käytätkö sen vuoksi lääkitystä? ei ( ) kyllä ( )
(vastaa tähän jos vastasit **kyllä** kysymykseen 127)





**Lapsuusaika**

**128.** Tupakoiko jompi kumpi vanhemmistasi tai joku samassa taloudessa asuvista kotonasi lapsuutesi aikana? ei ( ) äiti ( ) isä ( ) muu ( )

**129.** Oliko teillä kotona tai lähiympäristössä karvaisia lemmikkieläimiä kun olit alle viiden vuoden ikäinen? ei ( ) kyllä ( )

**130.** Onko Sinulla ollut jokin vakava hengitystieinfektio kun olit alle viiden vuoden ikäinen, esim. hinkuyskä tai keuhkokuume tai kurkunpääntulehdus? ei ( ) kyllä ( ) en tiedä ( )

**131.** Nukuitko yleensä samassa makuuhuoneen muiden lasten kanssa alle viiden vuoden ikäisenä? ei ( ) kyllä ( ) en tiedä ( )

**132.** Kuinka monta sisarusta Sinulla on /oli? _____

**133.** Kuinka monta **vanhempaa** sisarusta Sinulla on /oli? _____

**134.** Olitko päivähoidossa leikkikoulussa tai lastentarhassa muiden lasten kanssa kun olit alle viiden vuoden ikäinen? ei ( ) kyllä ( ) en tiedä ( )

**135.** Asuitko viiden ensimmäisen ikävuotesi aikana? kerrostalohuoneistossa ( ) omakoti-, rivi- tai paritalossa ( )

**136.** Missä asuit viiden ensimmäisen ikävuotesi aikana? maaseudulla ( ) taajamassa ( ) kaupungissa ( )

**Ammatti / työ**

**137.** Mikä on nykyinen työtilanteesi?

a) opiskelijana ( )
b) työssä ( )
c) työnhakijana ( )
d) varhaiseläkkeellä ( )
e) sairauseläkkeellä ( )
f) vanhuuseläkkeellä ( )
g) määräaikaiseläkkeellä/ sairauspäivärahalla(>6kk)( )
h) kotityössä ( )
i) muu ( )
j) asepalvelussa ( )
k) ei tietoa ( )
l) työttömyyseläkkeellä ( )
m)osaeläkkeellä ( )

**138.** Mikä on nykyinen tai viimeisin työsi?

_____ NYK: _____

SEI: _____

**139.** Onko Sinulla ollut muuta työtä, joka on kestänyt yli viisi vuotta?

ei ( )
kyllä ( )

vastaa kysymykseen 140 vain jos vastasit **kyllä** kysymykseen139:

140. Mikä työ / Mitä töitä?

_____ NYK: _____

SEI: _____

_____ NYK: _____

SEI: _____

**141.** Kuinka monta vuotta olet asunut nykyisessä asuinkunnassasi? _____vuotta

**142.** Kuinka monta vuotta olet asunut nykyisessä asunnossasi? _____vuotta



**Tupakan käyttö**

**143.** Oletko.....

ei koskaan polttanut (never-smoker) ( )
ei-tupakoitsija ( non-smoker ) ( )
entinen tupakoitsija ( )
nykyinen tupakoitsija ( )

**144.** Oletko koskaan tupakoinut yhtä vuotta? ( keskimäärin vähintäin yksi savuke päivässä tai ainakin yksi sikari viikossa tai vähintäin 30 grammaa piipputupakkaa kuukaudessa - yhteensä yhden vuoden ajan )

ei ( )
kyllä ( )

**145.** Kuinka vanha olit kun aloit tupakoida? _____vuotias

**146.** Altistutko tai oletko altistunut tupakansavulle kotiympäristössäsi?

ei ( )
kyllä, aiemmin, ei enää ( )
kyllä, vieläkin ( )

**147.** Altistutko tai oletko altistunut tupakansavulle työympäristössäsi?

ei ( )
kyllä, aiemmin, ei enää ( )
kyllä, vieläkin ( )

**148.** Altistutko yleensä toisten ihmisten tupakoinnille?

ei ( )
kyllä ( )

**149.** Oliko äitisi tupakoitsija raskausaikana kun hän odotti Sinua?

ei ( )
kyllä ( )
en tiedä ( )

**150.** Polttiko äitisi säännöllisesti Sinun varhaisen lapsuutesi aikana?

ei ( )
kyllä ( )
en tiedä ( )

**151.** Polttiko isäsi säännöllisesti Sinun varhaisen lapsuutesi aikana?

ei ( )
kyllä ( )
en tiedä ( )

**Entiset tupakoitsijat**

**152.** Kuinka vanha olit kun lopetit tupakoinnin? _____vuotias

**153.** Kuinka monta tupakkaa poltit keskimäärin päivässä ennen kuin lopetit ?

en yhtään ( )
1-4 ( )
5-14 ( )
15-24 ( )
≥ 25 ( )

**154.** Kuinka paljon piipputupakkaa poltit keskimäärin viikossa ennen kuin lopetit?
- en yhtään ( )
- < 50 g ( )
- 50 - 100 g ( )
- >100 g ( )

**Nykyiset tupakoitsijat**

**155.** Kuinka monta savuketta poltat keskimäärin päivässä?
- en polta ( )
- 0-4 ( )
- 5-14 ( )
- 15-24 ( )
- ≥ 25 ( )

**156.** Kuinka monta savuketta olet polttanut keskimäärin päivässä siitä alkaen kun aloitit tupakoinnin?
- en polta ( )
- 0-4 ( )
- 5-14 ( )
- 15-24 ( )
- ≥ 25 ( )

**157.** Jos poltat sikareja, kuinka monta poltat nykyisin keskimäärin päivässä?
- en polta ( )
- 0 - 1 ( )
- 2 - 4 ( )
- ≥ 5 ( )

**158.** Kuinka monta sikaria olet polttanut keskimäärin päivässä siitä lähtien kun aloitit?
- en polta ( )
- 0 - 1 ( )
- 2 - 4 ( )
- ≥5 ( )

**159.** Kuinka paljon piipputupakkaa käytät nykyisin keskimäärin viikossa?
- en käytä ( )
- < 50 g ( )
- 50 - 100 g ( )
- > 100 g ( )

**160.** Kuinka paljon piipputupakkaa olet käyttänyt keskimäärin viikossa siitä alkaen kun aloitit?
- en käytä ( )
- < 50 g ( )
- 50 - 100 g ( )
- > 100 g ( )

**161.** Oletko yrittänyt lopettaa tupakointia?
- ei ( )
- kyllä ( )

**162.** Oletko ollut välillä polttamatta?
- ei, tai yhteensä alle 1 vuoden ajan ( )
- kyllä, yhteensä _____ vuotta





**Keuhkofunktiotutkimus**

**163.** FVC paras kolmesta L _____
**164.** FVC normaaliarvo (ERS) L _____
**165.** FVC % normaaliarvosta % _____

**166.** $FEV_1$ paras kolmesta L _____
**167.** $FEV_1$ normaaliarvo (ERS) L _____
**168.** $FEV_1$ % normaaliarvosta % _____

**169.** EVC korkein arvo L _____

**170.** PEF L/min _____
**171.** PEF normaaliarvo (ERS) L/min _____
**172.** PEF % normaaliarvosta % _____

**173.** $MEF_{75}$ ($FEF_{25}$) L/min _____
**174.** $MEF_{75}$ ($FEF_{25}$) normaaliarvo (ERS) L/min _____
**175.** $MEF_{75}$ ($FEF_{25}$) % normaaliarvosta % _____

**176.** $MEF_{50}$ ($FEF_{50}$) L/min _____
**177.** $MEF_{50}$ ($FEF_{50}$) normaaliarvo (ERS) L/min _____
**178.** $MEF_{50}$ ($FEF_{50}$) % normaaliarvosta % _____

**179.** $MEF_{25}$ ($FEF_{75}$) L/min _____
**180.** $MEF_{25}$ ($FEF_{75}$) normaaliarvo (ERS) L/min _____
**181.** $MEF_{25}$ ($FEF_{75}$) % normaaliarvosta % _____

**182.** MMEF ($FEF_{25-75}$) L/min _____
**183.** MMEF ($FEF_{25-75}$) normaaliarvo (ERS) L/min _____
**184.** MMEF ($FEF_{25-75}$) % normaaliarvosta % _____

**185.** FVC paras kolmesta br.dil. testin jälkeen L _____

**186.** FEV1 paras kolmesta br.dil. testin jälkeen L _____

**Keuhkofunktiotutkimuksen raportti liitteenä**

**APPENDIX IV**

## The FinEsS Study - Intervjuformulär (2001-01-26 amended version)

Undersökningsdatum _____

Intervjuare _____

**Personuppgifter**

Namn _____

Adress _____

Telefonnummer hem _____

arbetet _____

**1.** Pnr _____

2. FinEsS nummer _____

**3.** Etnisk tillhörighet
- Kaukasier ( )
- Afrikan ( )
- Asiat/oriental ( )
- Annan ( )

**4.** Kön
- Man ( )
- Kvinna ( )

**5.** Urval
- Slumpurval ( )
- Stratifierat urval ( )

**6.** Landskod ___
Center kod ___
Områdeskod ___
Nationalitetskod ___

**7.** Tätortsgradient (ej i H:fors) ___



**Hosta och expektorat**

**8.** Har Du under de senaste åren haft långdragen hosta? Nej ( ) Ja ( )

**9.** Brukar Du hosta eller harkla Dig på morgonen? Nej ( ) Ja ( )

Om **JA** på fråga 9 besvaras fråga 10:

10. Kan sådan hosta eller harklingar förekomma de flesta dagar i perioder om mer än två veckor? Nej ( ) Ja ( )

**11.** Brukar Du hosta eller harkla Dig under andra tider på dygnet? Nej ( ) Ja ( )

Om **JA** på fråga 11 besvaras fråga 12:

12. Kan sådan hosta eller harklingar förekomma de flesta dagar i perioder om mer än två veckor? Nej ( ) Ja ( )

Om **JA** på fråga 10 eller 12 besvaras fråga 13:

13. Har Du denna hosta...
- enstaka perioder? ( )
- vanligtvis under vintertid? ( )
- alla dagar eller periodvis ( )
- under hela året? ( )

**14.** Brukar Du hosta eller harkla upp slem från bröstet? Nej ( ) Då och då ( ) Ofta ( )

**15.** Känner Du att det sitter slem i bröstet som Du har svårt att hosta eller harkla upp? Nej ( ) Ja ( )

**16.** Hostar eller harklar Du upp slem (eller har slem som det är svårt att få upp trots hosta) de flesta dagar i perioder om:
- Nej/inte alls/<3 mån/år ( )
- Minst 3 mån/år ( )
- Minst 3 mån/år under minst 2 påföljande år ( )

Om **JA** på alternativ tre på fråga 16 besvaras fråga 17:

17. Sedan hur många år? Antal år ____

Om **NEJ** på alternativ tre på fråga 16 besvaras fråga 18:

18. Har Du någonsin haft långdragen hosta eller långdragna perioder med slem i bröstet? Nej ( ) Ja ( )

**Pip i bröstet eller väsande andning**

**19.** Brukar Du ha pip, skräl eller väser det i bröstet då Du andas? Nej ( ) Ja ( )

**20.** Har Du någonsin, nu eller tidigare, vid något tillfälle haft pip eller väsningar i bröstet då Du andas? Nej ( ) Ja ( )

**21.** Har Du haft pip eller har det väst i bröstet vid något tillfälle under de senaste 12 månaderna? Nej ( ) Ja ( )

Om **JA** på fråga 21 besvaras frågorna 22-24 (25)

22. Har Du varit *det minsta* andfådd när Du haft pip eller väsningar i bröstet? Nej ( ) Ja ( )

23. Har Du haft detta pip eller väsande i bröstet när du *inte* samtidigt varit förkyld? Nej ( ) Ja ( )

24. Har Du pip i bröstet eller väsande andning de flesta dagarna i veckan? Nej ( ) Ja, periodvis ( ) Ja ( )

Om PERIODVIS på fråga 24, ange månad:

25. Månader med pip i bröstet eller väsande andning:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Januari | | Februari | | Mars | | April | | Maj | | Juni | |
| Juli | | Augusti | | September | | Oktober | | November | | December | |

Kan inte specificera ( )

**Andnöd**

**26.** Är Du rörelsehindrad av andra skäl än hjärt- eller lungbesvär? Nej/ej rel ( ) Ja ( )

Om **JA** på fråga 26 besvaras fråga 27:

27. Av vilka skäl? Cerebrovaskulär sjukdom ( ) Muskelsjukdom ( ) Rörelseinskränkning i extrem. ( ) Övrigt ( )

**28.** Är Du rullstolsbunden? Nej ( ) Ja ( )

**29.** Har Du någonsin besvär med din andning? Nej ( ) Ja ( )





Om **JA** på fråga 29 besvaras fråga 30:

30. Har Du dessa besvär För jämnan så att andningen aldrig är riktigt bra ( ) Återkommande men avlösta av besvärsfria perioder ( ) Endast vid enstaka tillfällen ( )

**31.** Besväras Du av andnöd eller andfåddhet när Du skyndar dig på plan mark eller i din egen takt går uppför en trappa eller mindre sluttning? Nej ( ) Ja ( )

Om **JA** på fråga 31 besvaras frågorna 32 - 34

32. Blir Du andfådd när Du går med andra personer i din egen ålder på plan mark? Nej ( ) Ja ( )

33. Måste Du stanna för att hämta andan när Du går på plan mark i Din egen takt? Nej ( ) Ja ( )

34. Blir Du andfådd då Du klär på eller av dig? Nej ( ) Ja ( )

**Anfall av andnöd eller trånghetskänsla i bröstet**

**35.** Har Du någonsin haft hastigt påkommande andnöd eller återkommande andfåddhet? Nej ( ) Ja ( )

**36.** Har Du någon gång under de senaste 12 månaderna haft hastigt påkommande andnöd eller andfåddhet? Nej ( ) Ja ( )

Om **JA** på fråga 35 eller 36 besvaras fråga 37:

37. Är din andning "normal" innan anfallen börjar eller mellan besvärsperioderna? Nej ( ) Ja ( )

**38.** Har Du någonsin haft hastigt påkommande andnöd med pip eller väsningar i bröstet? Nej ( ) Ja ( )

Om **JA** på fråga 38 besvaras fråga 39 och 40:

39. Har Du haft hastigt påkommande andnöd med pip och väsningar i bröstet under de senaste 12 månaderna? Nej ( ) Ja ( )

40. Hur gammal var Du när Du hade Din första episod eller anfall av andnöd? Ålder _____

**41.** Har Du någonsin vaknat nattetid eller tidigt på morgonen av anfall med andnöd och pip eller väsningar i bröstet? Nej ( ) Ja ( )

Om JA på fråga 41 besvaras fråga 42:

42. Har detta hänt under de senaste 12 månaderna? Nej ( )
Ja ( )

Om **JA** på fråga 36, 39 eller 42 besvaras fråga 43:

43. Hur många gånger har Du haft andnöd med eller utan pip i bröstet under de senaste 12 månaderna?

| | |
|---|---|
| Ingen | ( ) |
| Möjligen en | ( ) |
| Två men inte fler än fem | ( ) |
| Fler än fem men inte varje månad | ( ) |
| De flesta månader men inte varje vecka | ( ) |
| De flesta veckor men inte varje dag | ( ) |
| De flesta dagar | ( ) |
| Periodvis de flesta veckor | ( ) |
| Periodvis de flesta dagar | ( ) |

**44.** Har Du någonsin haft trånghetskänsla i bröstet? Nej ( )
Ja ( )

Om **JA** på fråga 44 besvaras fråga 45:

45. Har detta hänt under de senaste 12 månaderna? Nej ( )
Ja ( )

**46.** Har Du någon gång vaknat med trånghetskänsla i bröstet? (*ERSQ 5.E.1.1.2.4*) Nej ( )
Ja ( )

Om **JA** på fråga 46 besvaras fråga 47:

47. Har detta hänt under de senaste 12 månaderna? Nej ( )
Ja ( )

**Faktorer som framkallar pip eller väsningar i bröstet eller hastigt påkommande andnöd med eller utan hosta.**

**48.** Pälsdjurskontakt, t.ex. hund, katt, ko, häst, kanin etc? Nej ( )
Ja ( )

**49.** Pollenexponering, t.ex. lövsprickning, gräs, utomhusblommor? Nej ( )
Ja ( )

**50.** Mögellukt eller mögelexponering? Nej ( )
Ja ( )

**51.** Tobaksrök eller tobaksdoft? Nej ( )
Ja ( )

**52.** (ospecifikt) dammiga miljöer? Nej ( )
Ja ( )



**53.** Starka dofter som parfymer, kryddor, trycksvärta, stekos, rengöringsmedel, starkt doftande blommor etc? Nej ( )
Ja ( )

**54.** Bilavgaser? Nej ( )
Ja ( )

**55.** Yttre luftföroreningar, andra än bilavgaser? Nej ( )
Ja ( )

**56.** Infektioner, förkylningar? Nej ( )
Ja ( )

**57.** Läkemedel, t.ex. Magnecyl? Nej ( )
Om **JA**; vilka mediciner? Ja ( )
_____

**58.** Födoämnen som fisk, skaldjur, nötter, kärnförande frukter (parabjörkfenomen)? Nej ( )
Ja ( )

Om **JA**, vilken mat?
_____
_____

**59.** Psykiska faktorer eller stress? Nej ( )
Ja ( )

**60.** Kall luft? Nej ( )
Ja ( )

**61.** Någon annan speciell väderlek, t.ex. fuktig, blåsig, dimmig luft eller varm luft)? Nej ( )
Ja ( )

**62.** Får Du andfåddhet med pip i bröstet *direkt eller strax efter* fysisk ansträngning? Nej ( )
Ja ( )

**63.** Får Du andfåddhet med pip i bröstet *under* fysisk ansträngning? Nej ( )
Ja ( )

**64.** Har Du någon gång haft anfall av andnöd med pip eller väsningar i bröstet eller astmasymtom på din arbetsplats? Nej ( )
Ja ( )

Om **JA** på fråga 64 besvaras fråga 65

65. Har detta hänt under de senaste 12 månaderna? Nej ( )
Ja ( )

**66.** Får Du andfåddhet med pip i bröstet av något annat än vad som ovan nämnts? Nej ( )
Ja ( )
Om JA, specificera av vad? _____

## Astma och kronisk bronkit

**67.** Har Du eller har Du haft astma?
Nej ( )
Ja ( )
Vet ej ( )

**68.** Har Du av läkare fått diagnosen astma?
Nej ( )
Ja ( )
Vet ej ( )

**69.** Hade Du pip eller väsningar i bröstet i tidig barndom eller astma under barndomen?
Nej ( )
Ja ( )
Vet ej ( )

**70.** Använder Du eller har Du använt astmamediciner regelbundet eller vid behov?
Nej ( )
Ja ( )

**71.** Har Du av läkare blivit ordinerad astmaläkemedel?
Nej ( )
Ja ( )

Om **JA** på någon av frågorna 67 - 71 besvaras fråga 72 - 76:

72. Hur gammal var Du när Du av läkare fick diagnosen astma eller ordinerades astmaläkemedel?
Ålder _____

73. Hur gammal var Du när Du fick astma, astmasymptom eller ditt första astmaanfall?
Ålder _____

74. Hur gammal var Du när Du hade ditt senaste astmaanfall eller hade astmasymptom?
Ålder _____

75. Har Du överhuvudtaget haft några astmasymtom under de senaste 12 månaderna?
Nej ( )
Ja ( )

76. Har Du överhuvudtaget använt astmaläkemedel under de senaste 12 månaderna?
Nej ( )
Ja ( )

**77.** Har Du av läkare fått diagnosen kronisk luftrörskatarr eller emfysem?
Nej ( )
Ja ( )
Vet ej ( )

**78.** Anser Du själv att Du har kronisk luftrörskatarr?
Nej ( )
Ja ( )
Vet ej ( )

**79.** Anser Du själv att Du har emfysem?
Nej ( )
Ja ( )
Vet ej ( )





## Astmamediciner

Om JA på någon av frågorna 76 - 79 besvaras frågorna 80 - 93. Om inte annat anges gäller användning av astmamediciner under de senaste 12 månaderna.

80. Kortverkande beta-2 stimulerande medel i inhalationsform? (Ventoline, Salbuvent, Buventol, Bricanyl, Berotec)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

Om då och då eller de flesta dagar i veckan besvaras fråga 81:

*81.* Hur många år har Du använt kortverkande beta-2 stimulerare i inhalationsform?
Mindre än 1 år ( )
1 - 5 år ( )
Mer än 5 år ( )

82. Inhalationssteroider? (Becotide, Beclomet, Pulmicort,) Flixotide, Cortivent, Seretide)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

Om då och då eller de flesta dagar i veckan besvaras fråga 83 och 84:

*83.* Hur många år har Du använt inhalationssteroider?
Mindre än 1 år ( )
1 - 5 år ( )
> 5 år ( )

*84.* Vilken dos tar Du för närvarande?
≤ 200 mcg/dag ( )
>200 ≤ 800 mcg/dag ( )
> 800 mcg/dag ( )

85. Antikolinergika i inhalationsform? (Atrovent, Atrovent comp., Ventox)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

86. Natriumkromoglikat i inhalationsform? (Lomudal, Tilade)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

87. Långverkande beta-2-stimulerare i inhalationsform? (Serevent, Foradil, Oxis, Seretide)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

88. Beta-2-stimulerande medel i tablettform? (Ventoline, Salbuvent, Bricanyl)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

89. Theofyllin? (Euphyllin, Retafyllin, Theophyllin, Aminocont, Theo-Dur, Nuelin)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

90. Luftvägsvidgande medel i form av inhalations- vätska genom inhalationsapparat? (Ventoline, Bricanyl, Atrovent)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )

91. Perorala steroider? (t.ex Medrol, Prednisolon)
Nej ( )
Då och då ( )
De flesta dagar i veckan ( )
Endast vid luftvägs-exacerbationer ( )

Om **NEJ** på fråga 91 besvaras fråga 92:

*92.* Har Du tidigare använt perorala steroider?
Nej ( )
Ja ( )

Om **JA** på fråga 92 besvaras fråga 93:

*93.* Varför slutade Du?
Började med inhalationssteroider ( )
Förbättrad ( )
Annan orsak ( )
Endast vid luftvägsexacerbationer ( )

**Hostmediciner**

**94.** Använder eller har Du tidigare använt host- eller slemlösande medicin oftare än sporadiskt vid vanlig förkylning?
Nej ( )
Ja ( )

Om JA på fråga 94 besvaras fråga 95 och 96:

95. Acetylcystein? (Mucomyst, Mucoporetta)
Nej ( )
Ja ( )

96. Annan slemlösande eller hostdämpande medicin?
Nej ( )
Ja ( )

**Mediciner mot rhinit och konjunktivit**

**97.** Använder eller har Du tidigare använt medicin mot rhinit eller konjunktivit?
Nej ( )
Ja ( )

Om JA på fråga 97 besvaras fråga 98-100:

98. Antihistaminer i tablettform? (t.ex Teldanex, Semprex, Disofrol, Duact, Kestine, Hismanal, Lunerin,) Clarityn, Cirrus, Rinomar, Zyrtec)
Nej ( )
Ja ( )

99. Nasala steroider? (t.ex Beconase, Rhinocort, Flixonase, Lokilan Nasal Becotide Nasal, Beclonasal)
Nej ( )
Ja ( )



100. Övrigt (inkl Lomudal) för näsa eller ögon?
Nej ( )
Ja ( )

**Sjukvårdsbehov**

**101.** Har Du överhuvudtaget haft några problem med din andning eller besvär av hosta eller slem?
Nej ( )
Ja ( )

**102.** Har Du någonsin sökt läkare eller sjukvård pga andfåddhet, andnöd eller pip i bröstet?
Nej ( )
Ja ( )

**103.** Har Du någonsin sökt läkare eller sjukvård pga långdragen hosta, slemhosta eller slem i bröstet?
Nej ( )
Ja ( )

Om **JA** på fråga 101, 102 eller 103 besvaras fråga 104 -115

104. Har Du sökt läkare *under de sista 12 månaderna* för att Du haft problem med Din andning eller besvär av hosta eller slem?
Nej ( )
Ja ( )

Om JA på fråga 104 besvaras fråga 105:

105. Hur många gånger har Du sökt läkare under de sista 12 månaderna för att Du haft problem med din andning eller besvär av hosta eller slem? Antal gånger_____

106. Går Du på regelbundna kontroller för luftvägs- eller andningsproblem?
Nej ( )
Ja ( )

107. Har Du på grund av andningsproblem eller besvär av hosta eller slem sökt specialistläkare i lungmedicin eller eller allergologi?
Nej ( )
Ja ( )

Om JA på fråga 107 besvaras fråga 108:

*108.* Utfördes ett prick-test?
Nej ( )
Ja ( )

Om JA på fråga 108 besvaras fråga 109:

*109.* Var prick-testet positivt?
Nej ( )
Ja ( )

110. Har Du någon gång behövt uppsöka akutmottagning på grund av andnings besvär?
Nej ( )
Ja ( )

111. Har Du någon gång varit inlagd på sjukhus för andningsbesvär?
Nej ( )
Ja ( )

112. Är Du nöjd med den hjälp Du fått från "sjukvården" för Dina luftvägsbesvär?
Nej ( )
Ja ( )
Ej relevant ( )

113. Upplever Du att andnöd, tungandning eller pip i bröstet påverkar Dig i Ditt dagliga liv?
Nej ( )
Då och då ( )
Ofta ( )

114. Upplever Du att hosta eller slembildning påverkar Dig i Ditt dagliga liv?
Nej ( )
Då och då ( )
Ofta ( )

115. Hur påverkas Ditt dagliga liv av dina besvär från luftvägarna?
Inte alls ( )
Något ( )
Ibland måttligt ( )
Måttligt ( )
Mycket ( )

**Övriga sjukdomar**

**116.** Har Du eller har Du haft någon annan lung/luftvägssjukdom än astma, kronisk luftrörskatarr eller emfysem?
Nej ( )
Ja ( )

Om JA på fråga 116 besvaras fråga 117:

117. Vilken/vilka?

_____

_____

_____

**118.** Har Du haft tbc?
Nej ( )
Ja, lungtbc ( )
Ja, annan tbc ( )

**119.** Har eller har Du haft hösnuva, allergisk rinnsnuva eller konjunktivit?
Nej ( )
Ja ( )

**120.** Besväras Du ofta av nästäppa eller rinnande snuva?
Nej ( )
Ja ( )

**121.** Har Du eller har Du haft näspolyper?
Nej ( )
Ja ( )

**122.** Har Du eller har Du haft (även som barn) eksem?
Nej ( )
Ja ( )



**123.** Har Du eller har Du haft hjärtproblem eller hjärtsjukdom?
Nej ( )
Ja, kärlkramp ( )
Ja, hjärtinfarkt ( )
Ja, hjärtsvikt ( )
Ja, rytmrubbning ( )
Ja, annan hjärtsjukdom ( )

**124.** Använder Du hjärtmediciner?
Nej ( )
Ja, en medicin ( )
Ja, två mediciner ( )
Ja, tre mediciner ( )
Ja, fyra mediciner ( )
Mer än fyra mediciner ( )

**125.** Har Du eller har Du haft högt blodtryck?
Nej ( )
Ja ( )

**126.** Använder Du någon medicin mot högt blodtryck?
Nej ( )
Ja ( )

**127.** Har Du någon annan icke tidigare nämnd sjukdom?
Nej ( )
Ja ( )

**Uppväxttid**

**128.** Rökte någon av dina föräldrar eller någon annan i Din hemmiljö under Din uppväxttid? (före skolåldern)
Nej ( )
Mor ( )
Far ( )
Annan anhörig ( )

**129.** Hade ni pälsdjur i hemmiljön eller i den nära omgivningen när Du var yngre än fem år?
Nej ( )
Ja ( )

**130.** Hade Du någon allvarlig luftrörs- eller lunginfektion före fem års ålder, t ex kikhosta eller krupp?
Nej ( )
Ja ( )
Vet ej ( )

**131.** Brukade Du dela sovrum med andra barn före fem års ålder?
Nej ( )
Ja ( )
Vet ej ( )

**132.** Hur många syskon har Du eller har Du haft?
Antal _____

**133.** Hur många äldre syskon har Du eller har Du haft?
Antal _____

**134.** Vistades Du på daghem, lekskola eller barnhem tillsammans med andra barn före fem års ålder?
Nej ( )
Ja ( )
Vet ej ( )

**135.** Hur bodde Du under dina första fem levnadsår?
Villa ( )
Lägenhet ( )

**136.** Var bodde Du under Dina första fem levnadsår?
Landsbygd ( )
Förort ( )
Stad/tätort ( )

**Yrke/arbete**

**137.** Vilket är Ditt nuvarande yrke/sysselsättning?
Studerar ( )
Arbetar ( )
Arbetssökande ( )
Förtidspension ( )
Sjukpension ( )
Ålderspension ( )
Långtidssjukskriven (mer än 6 månader) ( )
Hemarbetande ( )
Övrigt ( )
Militärtjänst ( )
Okänt ( )

**138.** Vad är Ditt nuvarande eller senaste yrke?

_____ NYK: ____
SEI: ____

**139.** Har Du arbetat mer än 5 år i något annat yrke?
Nej ( )
Ja ( )

Om JA på fråga 139 besvaras fråga 140:

140. Vilket yrke/yrken?

_____ NYK: ___
SEI: ___

_____ NYK: ___
SEI: ___

**141.** Hur många år har Du bott i Din nuvarande kommun? Antal år ___

**142.** Hur många år har Du bott i Din nuvarande bostad? Antal år ___



**Rökning och nikotin användning**

**143.** Är Du...
Aldrig-rökare ( )
Icke rökare ( )
(slutat >12 mån. sedan) Före detta rökare ( )
Rökare (>1cig/vecka) ( )

**144.** Har Du någonsin rökt minst ett år? (Minst en cigarett/dag - minst en cigarr/vecka eller minst 30 gram tobak/månad - under minst ett års tid)
Nej ( )
Ja ( )

Om **JA** på fråga 144 besvaras fråga 145:

145. Hur gammal var Du när Du började röka? Ålder ____

**146.** Är Du eller har Du varit utsatt för rökning i Din hemmiljö?
Nej ( )
Ja tidigare, ej nu ( )
Ja, även nu ( )

**147.** Är Du eller har Du varit utsatt för rökning på din arbetsplats?
Nej ( )
Ja tidigare, ej nu ( )
Ja, även nu ( )

**148.** Utsätts Du ofta för andra människors tobaksrök?
Nej ( )
Ja ( )

**149.** Rökte Din mor då hon var gravid med dig?
Nej ( )
Ja ( )
Vet ej ( )

**150.** Rökte din mor regelbundet under din tidiga barndom?
Nej ( )
Ja ( )
Vet ej ( )

**151.** Rökte din far regelbundet under din tidiga barndom?
Nej ( )
Ja ( )
Vet ej ( )

**Före detta rökare (frågorna besvaras av före detta rökare)**

152. Hur gammal var Du när Du slutade att röka? Ålder ____

153. Hur många cigaretter per dag rökte Du i genomsnitt under hela den tidsperiod Du var rökare?
Rökte ej ( )
1 - 4 ( )
5- 14 ( )
15- 24 ( )
25 eller mer ( )

154. Hur mycket piptobak rökte Du i genomsnitt per vecka under hela den tidsperiod Du var rökare?
- Rökte ej ( )
- < 50 g ( )
- >50 - <100 g ( )
- > 100 g ( )

**Rökare (frågorna besvaras av rökare)**

155. Om Du röker cigaretter, hur många röker Du i genomsnitt per dag?
- Röker ej ( )
- 1 -4 ( )
- 5 - 14 ( )
- 15 - 24 ( )
- 25 eller fler ( )

156. Hur många cigaretter per dag har Du i genomsnitt rökt sedan Du började röka?
- Röker ej ( )
- 1 - 4 ( )
- 5 - 14 ( )
- 15 - 24 ( )
- 25 eller fler ( )

157. Om Du röker cigarrer, hur många röker Du i genomsnitt per dag?
- Röker ej cigarrer ( )
- 0 - 1 ( )
- 2 - 4 ( )
- 5 - > ( )

158. Hur många cigarrer per dag har Du i genomsnitt rökt sedan Du började röka?
- Röker ej cigarrer ( )
- 0 - 1 ( )
- 2 - 4 ( )
- 5 - > ( )

159. Om Du är piprökare, hur mycket förbrukar Du i genomsnitt per vecka?
- Röker ej pipa ( )
- < 50 g ( )
- > 50 - <100 g ( )
- > 100 g ( )

160. Hur mycket piptobak per vecka har du i genomsnitt förbrukat sedan Du började röka?
- Röker ej pipa ( )
- < 50 g ( )
- > 50 - < 100 g ( )
- > 100 g ( )

161. Har Du försökt sluta röka?
- Nej ( )
- Ja ( )

162. Har Du gjort uppehåll från rökningen?
- Nej/, mindre än ett år ( )
- Ja, 1 - 5 år ( )
- Ja, mer än 5 år ( )





**Lungfunktionsprov**

| | | | |
|---|---|---|---|
| **163** | FVC bäst av tre | l | ____ |
| **164** | FVC normalvärde | l | ____ |
| **165** | FVC % av normalvärde | % | ____ |
| **166** | $FEV_1$ bäst av tre | l | ____ |
| **167** | $FEV_1$ normalvärde | l | ____ |
| **168** | $FEV_1$ % av normalvärde | % | ____ |
| **169** | EVC högsta värde | l | _____ |
| **170** | PF | l/min | _____ |
| **171** | PF normalvärde | l/min | _____ |
| **172** | PF % av normalvärde | % | ____ |
| **173** | MF75 (FF25) | l/min | _____ |
| **174** | MF75 (FF25) normalvärde | l/min | _____ |
| **175** | MF75 (FF25) % av normalvärde | % | ____ |
| **176** | MF50 (FF50) | l/min | _____ |
| **177** | MF50 (FF50) normalvärde | l/min | _____ |
| **178** | MF50 (FF50) % av normalvärde | % | ____ |
| **179** | MF25 (FF75) | l/min | _____ |
| **180** | MF25 (FF75) normalvärde | l/min | _____ |
| **181** | MF25 (FF75) % av normalvärde | % | ____ |
| **182** | MMF (FMF) | l/min | _____ |
| **183** | MMF (FMF) normal value | l/min | _____ |
| **184** | MMF (FMF) % of normal value | % | ____ |
| **185** | FVC efter rev.test bästa värde av tre | l | ____ |
| **186** | $FEV_1$ efter rev.test bästa värde av tre | l | ____ |

**Lungfunktionskurva**